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The head Skeleton of the Lizard

by

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Introduction

The head skeleton of the lizard is reviewed because of the need to know more clearly and concisely the variations of structure within this type in order to compare it with other types of reptiles, birds and mammals. This review is a part of a survey of vertebrate head skeletons which has the ultimate goal of better understanding the evolution (and therefore homologies) of the individual elements in the several types of vertebrates.

There has been considerable discussion of homology but generally the comparisons upon which such discussions have been based are of a limited type: either between widely separated types (i.e., bird and mammal, frog and lizard) or closely related forms showing little variations. This study is based on the assumption thas by assessing the range of variation of morphological design in each of the classes of vertebrates one can then more accurately extrapolate from one class to the next, beginning with the mammals (the source of names) and working in a retrophylogenetic direction. The validity of extrapolation is based on the theory that evolution has occurred (at least basically) by micromutations and that changes in structure are usually of a functional and adaptive nature. Further it is believed that a morphological feature is the result of a very complex interplay of factors during development and the course (stages of development) or the end product can vary from individual to individual as well as in the various taxonomic catagories. Thus the end results of development can range from "exact" correspondence to scarcely any or no correspondence. It is an assumption of this report that if any "real" relationship exists between structures in two classes of vertebrates they are homologs; if no phylogenetic tie-up can be demonstrated then

they are not homologs. An example of the results of this type of approach is the correspondence of certain bones of the lower jaw of the reptile with the malleus and incus of the mammal. The phylogenetic histories of the stapes and of the alisphenoid are other examples.

Through this approach it is believed that the head skeleton of the tetrapod has shown marked constancy in the number and interrelationships of its osseous units since the Devonian. These primordial units can be identified with terms used in describing the human skull. Many problems remain regarding the structure of the head skeleton but since these have been "solved" in all possible ways it becomes a matter of selecting the solution which appears to agree best with the facts, as far as these can be determined. There are many differences of opinion to be reconciled and much confusion due to terminology to be eliminated before a clarification of the general morphology of the head skeleton in each vertebrate class can result.

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This paper is an attempt to describe the lizard skull using the terms of mammalian anatomy (as far as is meaningful). It is based on a retrophylogenetic exploration of the anatomy (developmental and mature) of head skeletons from the mammal to the reptile. It does not presume to describe every feature nor every detail of difference but it does attempt to give a working knowledge of the limits of variation of the major features, those which might be found in the head skeletons of other orders of reptiles or other classes of vertebrates. Whereas DE BEER ('37) assumed that comparisons of the chondrocrania would be most revealing as to phylogeny, emphasis here is on the osteocranium and adjacent bones.

Although the descriptive accounts of the lizard head skeleton are fairly limited in number, this literature is too bulky for easy review; further, the various papers are scattered in many journals and thus not readily available. The prime purposes of this report are, therefore, to facilitate study of this group of animals by bringing together some of the facts contained in this literature and to check the reports against actual specimens. It is also desireable to illustrate thoroughly another species of lizard, although many excellent figures are already available for several species.

Many details of the lizard skull have been described as a part of a catalogue of lizards prepared by BOULENGER (1885—87), some of these are still referred to since further anatomical investigation has not taken place (Dibamidae). A large part of our knowledge of the head skeleton of this group can be traced back to SIEBENROCK (1892—1895) who compared agamids, geckos, scincids, anguids, gerrhosaurids and lacertids. His terminology of the bones of the endocranium has been largely changed but he is still quoted in regard to many details. COPE (1892) attempted the first really systematic review of the structure of the lacertilian head skeleton since Cuvier. His review has been much criticised but still serves as a starting point. Many aspects of the head skeleton were discussed by CAMP ('23)

as a part of a comparative study of lizards. In a more general way the comparative anatomy of this type of skull was discussed by VERSLUYS ('36). The most recent summary is that of ROMER ('56) in his "Osteology of the Reptiles."

The materials of a comparative anatomy of this type are primarily detailed reports on individual species or small groups of species. The latter type is exemplified by the papers of SIEBENROCK (1892—1895), KINGMAN ('32), WELLBORN ('33), MERTENS ('42), ZANGERL ('44) and MCDOWELL and BOGERT ('54). The most recent example of the former is the study of *Ctenosaura pectinata* by OELRICH ('56). Cranial osteology forms only a part of Oelrich's study which interrelates details of bone, muscle, nerve and blood vessel. Other detailed studies of cranial anatomy, often based on sectioned material, include RAO and RAMASWAMI ('52, the scincid *Mabuya*), TOERIEN ('50, *Anniella*), VAN PLETZEN ('46, *Cordylus*), KRITZINGER ('45, the amphisbaenid *Monopeltis*), BROCK ('40—50, the scincid *Acontias*), MALAN ('40, *Gerrhosaurus*), BAHL ('37, *Varanus*), and HAAS ('35, '36, the scincids *Ablepharus* and *Chalcides*). LAKJER ('27) summarized much of the very early descriptive literature in his review of palatal structure in lizards.

Study of the head skeleton of any class of vertebrates should be based on developmental information. PARKER was the first to describe developmental stages (1879, *Lacerta;* 1880—85, chameleons). His hand-cut sections did not reveal the many details needed for a comprehensive, reliable understanding. These were largely supplied by GAUPP'S ('00) model of *Lacerta agilis.* Gaupp's study was followed by those of RICE ('20, *Eumeces*), PEARSON ('21, *Lygosoma*), BROCK ('32, *Lygodactylus* and *Pachydactylus;* '40, *Microsaura*), RAMASWAMI ('46, *Calotes*), and DERANIYAGALA ('58, *Varanus).* Additional references are given by DE BEER ('37) and LAKJER ('27).

The basic materials of this account are two partly cleaned head skeletons of *Tupinambis nigropunctatus* purchased from the Carolina Biological Supply House. This species was described by REESE ('23) and figured by VERSLUYS ('36, Figs. 563, 564, 573A and B, 598). A fresh specimen of *Tupinambis nigropunctatus* and preserved specimens of *Chamaeleo (dilepis?)* and *Amphisbaena alba* were dissected to observe the relationship of soft parts to hard parts. Specimens of several other genera were partly dissected to check specific details. Skeletal materials of many species were examined at the Carnegie Museum of Pittsburgh. Table 1 indicates the specimens examined as well as the more detailed literature sources for each genus. The generic names used here are those of ROMER ('56). The materials in some instances consist only of figures. Those of MCDOWELL and BOGERT ('54) have been particularly useful.

I want to thank Mr. Neil D. Richmond for making the materials under his care at the Carnegie Museum available for study.

Skull

The skull is made up of bones of dermal and cartilaginous origin and includes, in this case, the bones enclosing the brain (osteocranium) and those of the upper jaw. In this account little attention is given to whether a bone is chondral or dermal in origin; this information is readily available from DE BEER's ('37) account of *Lacerta*.

Basioccipital (Figs. 1—6, 9, 12)

The basioccipital forms the posterior floor of the cranium between the otic capsules. It contributes to the occipital condyle which is usually formed in part from the proatlas (not in *Gekko gekko*). Laterally the basioccipital contacts and is fused with the exoccipitals; a suture line may remain. Anteriorly the basioccipital contacts the basisphenoid but is separated from it usually by a distinct suture. In *Cordylus polyzonus* (but not *C. giganteus*—VAN PLETZEN, '46, Fig. 2; MCDOWELL and BOGERT, '54, Fig. 26), *Pygopus lepidopus* (LAKJER, '27, Fig. 20, but not in MCDOWELL and BOGERT, '54, Fig. 17), *Delma fraseri* (MCDOWELL and BOGERT, '54, Fig. 19), and also (?) *Calotes jabutus* (LAKJER, '27, Fig. 8a) there is a basicranial fontanelle between the basioccipital and basisphenoid; in *Chamaesaura* (VAN PLETZEN, '46: 58) there is a double opening here. A minute opening here can occur in many species as a variant (*Heloderma*). The basioccipital is very thin in this region or just behind this suture because of the basicranial fenestra of the chondrocranium.

The basioccipital gives rise to lateral processes (tuberculum sphenooccipitale) which may be well developed (*Tupinambis, Uromastix, Gekko*) or low and rounded (*Heloderma,* chameleons and burrowing forms). The tip of this process may have one or two (*Uromastix*) bony epiphyses attached to it. Where the processes are not large, there may be a distinct ossicle with interdigitating sutures separating it from the surrounding bones (basioccipital, basisphenoid, prootic—*Varanus*) or a simple but large epiphysis (*Heloderma, Amphisbaena*—Fig. 9). *Mabuya* and *Lygosoma* lack even a cartilaginous epiphysis (RAO and RAMASWAMI, '52: 258). These processes are very low and massive ridges in amphisbaenids and the epiphyseal ossicles are large (the X ossicles of ZANGERL, '44); the ossicles are the site of attachement of the powerful *longus capitis* muscles.

The lateral aspect of the basioccipital process is sometimes largely occupied by the recessus scalae tympani (SIEBENROCK, 1894: 211; ROMER, '56: 118); this is the case in *Anolis, Ctenosaura, Ophisaurus, Cnemidophorus* and *Heloderma.* This recess is intermediate in size in *Eumeces, Mabuya,* or *Gekko;* it is small in *Varanus, Uromastix, Tupinambis* and *Anguis.* The outer opening of this recess is the fenestra cochleae (rotunda), the inner opening is a remnant of the fissura meto-

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Head Skeleton of Lizard

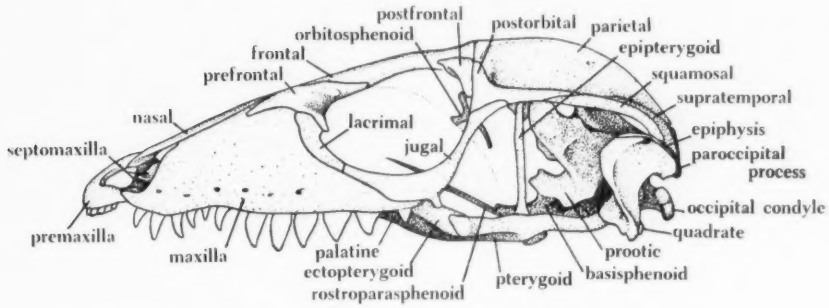

Fig. 1. Lateral view of the skull of *Tupinambis nigropunctatus*.

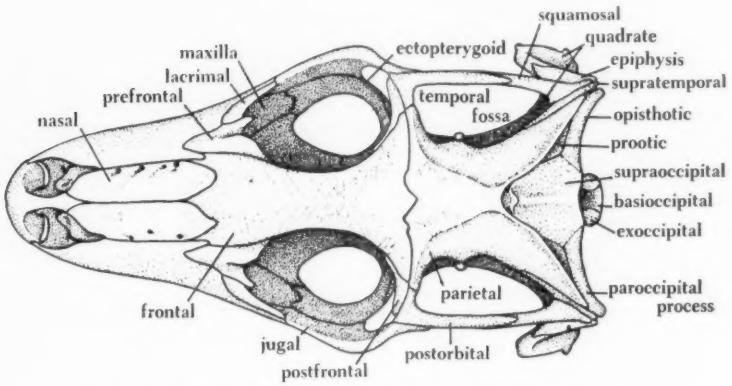

Fig. 2. Dorsal view of the skull of *Tupinambis nigropunctatus*.

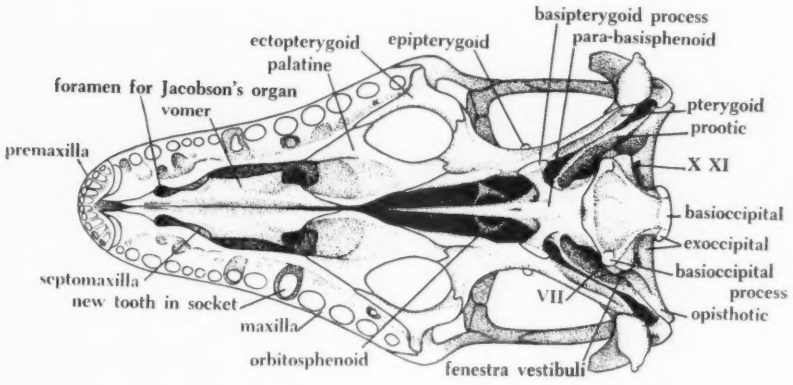

Fig. 3. Ventral view of the skull of *Tupinambis nigropunctatus*.

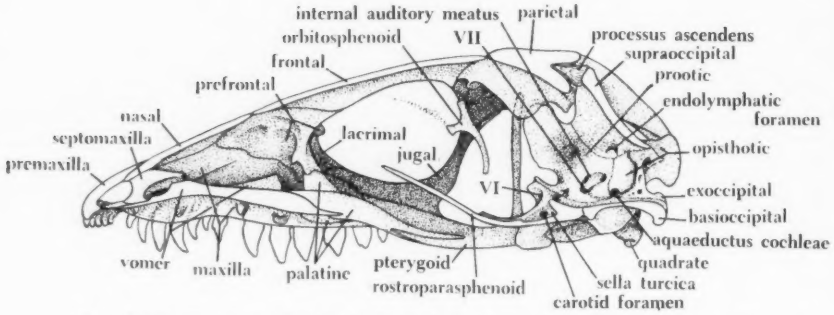

Fig. 4. Medial view of right half of skull of *Tupinambis nigropunctatus*.

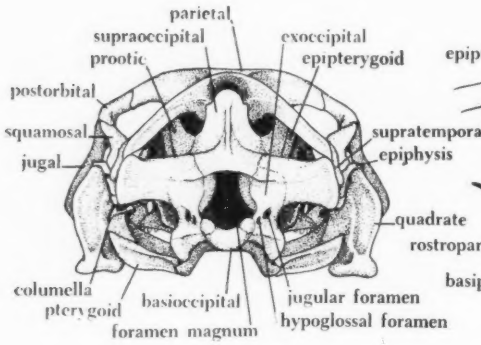

Fig. 5

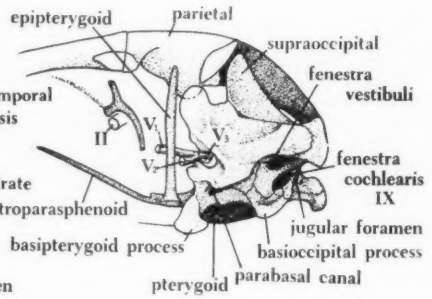

Fig. 6

Fig. 5. Posterior view of skull of *Tupinambis nigropunctatus*. Columella shown on left side *only*.

Fig. 6. Lateral view of cranium of *Tupinambis nigropunctatus* with temporal arch, quadrate and pterygoid removed.

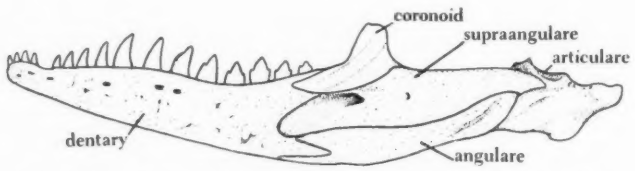

Fig. 7. Lateral view of mandible of *Tupinambis nigropunctatus*.

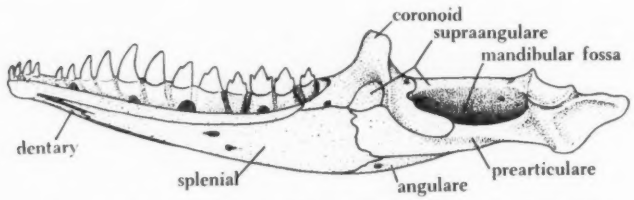

Fig. 8. Medial view of mandible of *Tupinambis nigropunctatus*.

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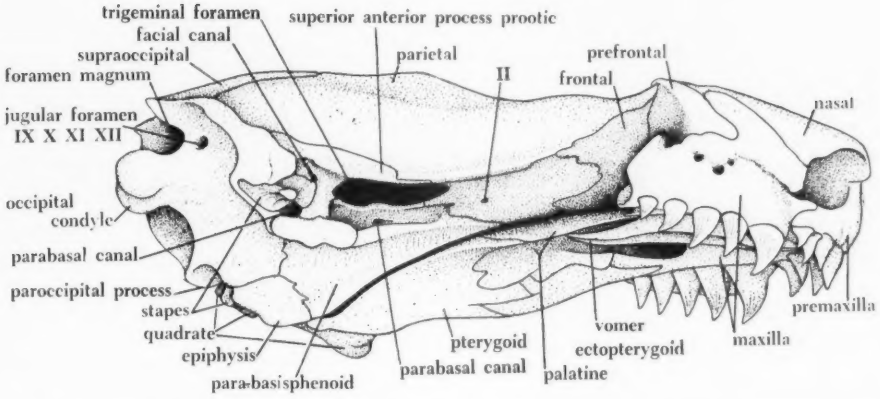

Fig. 9. Ventrolateral view of skull of *Amphisbaena alba* with the quadrate, pterygoid, palatine and ectopterygoid of the right side removed.

tica (aquaeductus cochleae) through which the perilymphatic sac (insipient scala tympani) projects into the cranial cavity. The fenestra cochleae is usually ovoid in shape, the long axis nearly vertical or with a dorsoposterior cant; in *Gekko* it is nearly round. This fenestra is margined anteriorly and dorsally by the opisthotic, posteriorly by the exoccipital and ventrally by the basioccipital. This fenestra is lacking in *Amphisbaena alba* and *Monopeltis* (KRITZINGER, '45), the entire region being covered by the foot-plate of the stapes. It is small (or lacking—PARKER, 1880—85: 102) in *Chamaeleo.*

The foramen metotica (aquaeductus cochleae; foramen perilymphaticus of OELRICH, 56; VERSLUYS, '36) which opens between the opisthotic and exoccipital may be very large (*Anolis, Ophisaurus, Cnemidophorus*) or quite small (*Eumeces, Tupinambis*). There is an opening in the top of the recessus leading into the cavum cochlearis (next to the lagena of the membranous inner ear); this is the foramen perilymphaticum of RAO and RAMASWAMI ('52), the internal perilymphatic foramen of this account.

Exoccipital (Figs. 1—6, 9, 12)

The bilateral exoccipitals lie to either side of the foramen magnum, meeting dorsally the (epiotic and) supraoccipital, ventrally the basioccipital and anteriorly the prootic. The exoccipital arises in the occipital arches of the cranial vertebrae (posterior to the metotic fissure) and fuses almost immediately and completely (no suture line) with the opisthotic; the combined bone can be called the otoccipital. Fusion of the exoccipital and the opisthotic is observed in the bird and in reptiles generally although a suture line may delimit these bones even in the adult. In *Sphenodon* such a suture persists only until full growth is achieved. In mam-

mals the periotic ossifications remain separate or fuse only secondarily with the occipital ring of bones.

The exoccipital is penetrated just above its ventral (basioccipital) margin by the one, two (usually) or three divisions of the hypoglossal (XII) nerve. The number of foramina on the inside may be one or two more than on the outside, the more anterior divisions emerging with nerves X and XI from the external jugular foramen.

The inner opening of the jugular canal represents the more dorsal part of the fissura metotica of the chondrocranium; the aquaeductus cochlae (foramen metotica) marks the ventral anterior part. The outer opening of the jugular canal is through the exoccipital and it may be confluent with (above) the exit or exits of the anterior divisions of the hypoglossal or with the entire hypoglossal compliment (*Chamaeleo, Varanus, Heloderma, Lanthanotus, Amphisbaena*).

The glossopharyngeal nerve usually exits through the metotic canal (through the fenestra cochlearis). It may have a separate internal foramen (*Lygosoma*—Pearson, '21: 23; *Mabuya*—Rao and Ramaswami, '52, Fig. 16; *Gekko*—Versluys, '36, Fig. 580E), or a separate external foramen through the exoccipital near or with the external jugular foramen (*Chamaeleo, Amphisbaena fuliginosa*—Versluys, 1898, Figs. 72, 83; *Monopeltis*—Kritzinger, '45: 190): In the lizard the external anterolateral boundary of the exoccipital is marked by the posterior rim of the fenestra cochleae (the tympanic crest—the crista tuberalis of Oelrich, '56); the internal anterior limit is marked by the crescentic jugular foramen and the round aquaeductus cochleae.

Posteriorly the exoccipital forms part of the occipital condyle. The suture between exoccipital and basioccipital is usually detectable at least on the condyle (not in *Anolis, Scincus, Lygosoma, Amphisbaena*). El-Toubi ('38: 6, apparently swayed by Kingsley and quoted by Rao and Ramaswami, '52: 258) stated that in *Scincus* the occipital condyle is presumed to be entirely basioccipital; however, despite lack of suture lines and the narrowness of the condyle it is probably like that of other lizards. In *Chamaeleo* the large exoccipital condylar components meet above the small basioccipital contribution. The condyle has a cartilaginous epiphyseal cover.

The suture between exoccipital and supraoccipital is observed in some species (*Ctenosaura, Chamaeleo, Varanus, Anguis*) but not in others (*Sceloporus, Phrynosoma, Conolophus, Iguana, Tupinambis, Cnemidophorus, Scincus, Eumeces, Heloderma, Ophisaurus, Amphisbaena*). Fusion is usually a function of age, the sutures being distinct in the young of all.

Supraoccipital (Figs. 1, 2, 4—6, 9, 12)

The supraoccipital forms the dorsal margin of the foramen magnum and sutures or fuses laterally with the exoccipitals. Its anterior margin bears a median

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cartilaginous (sometimes calcified—*Lygosoma*) processus ascendens (processus anterior tecti) which fits in a socket of the much thickened posterior margin of the parietals. The anterior margin of the bone is usually separated by a connective tissue gap from the parietal. In some burrowers—amphisbaenids, skinks (*Feylinia, Anelytropsis*) and *Anniella*—there is a suture except in the region of the processus ascendens where a small gap persists (no gap in *Rhineura*—ZANGERL, '44, Fig. 15). In the burrowing skink *Acontias*, the supraoccipital and parietals are fused (BROCK, '41: 79).

In the cordylids a process of the supraoccipital articulates with the posterior extension or medial forked process of the parietal. Similarly the posterior extension of the parietals in *Xantusia* (and ? *Phrynosoma, Conolophus,* etc), rests on a dorsal process of the supraoccipital. Such contacts reduce or eliminate movement along the occipito-parietal joint (reduce metakinesis).

In *Amphisbaena* the supraoccipital forms the posterior continuation of the sagittal crest of the parietals. The dorsal margin of the foramen magnum is the posterior margin of the cranium and the medial part of a nuchal crest which extends out to the paroccipital processes of the opisthotics. In most lizards the supraoccipital lies well below the level of the parietal and its dorsal outline slopes down from the processus ascendens to the dorsal margin of the foramen magnum, which does not lie on a "nuchal crest." There is usually a slight dorsal sagittal crest on the supraoccipital; such a crest is poorly marked or lacking in geckos, pygopodids, some scincids (*Chalcides, Voeltzkowia*), most diploglossans and *Anniella*. It may be present or absent in *Varanus* (*V. niloticus* has crest). MCDOWELL and BOGERT ('54: 104) used the lack of such a crest as distinctive of the anguimorphs; however, *Ophisaurus attenuatus* has a crest and is not unlike *Varanus niloticus*, or one of the skink, in this feature.

The taenia marginalis calcifies in the large iguanids (*Iguana, Conolophus, Amblyrhynchus, Ctenosaura*) in the region of its attachment to the otic capsule, at the anterior lateral margin of the supraoccipital. This calcification extends forward just inside the descending process of the parietal. There may be some calcification here in other lizards. *Chamaeleo* has a long but narrow bony process, the marginal process of OELRICH ('56), which lies distinctly medial to the lateral margin.

The supraoccipital arises from a single center in the synotic tectum or tectum posterior. It fuses almost immediately with bilateral epiotic centers of the ear capsule. ROMER ('56: 66) has suggested that the supraoccipital is but a derivative of these otic capsule centers. In the mammal the occipital ring has achieved a large or a complete measure of freedom from the otic capsules suggesting that the supraoccipital is indeed a primordial bone; presence of this bone in coelacanths (crossopterygian fishes) supports the contention.

Prootic (Figs. 1—6, 9, 12)

The prootic forms much of the lateral wall of the cranium, medial to the temporal musculature. It encloses the ampullae and much of the anterior and horizontal semicircular canals, and the anterior and dorsal parts of the utriculus, sacculus, and lagena (see ROMER, '56, Fig. 23, inner ear of *Lacerta;* OELRICH, '56, Figs. 54—56, of *Ctenosaura;* CAMP, '42, Fig. 26, of *Varanus*). The prootic forms the anterior dorsal margin of the fenestra vestibuli. It has a large paroccipital process which extends out nearly to the tip of that opisthotic process (but does not contribute to the articular area of the quadrate). The anterior margin of this bone is usually notched (incisura prooticum) at about the level of the anterior ampulla. Through this notch pass the fifth nerve and the vena cerebralis media (latter above former). Below the notch is an inferior anterior process which joins the clinoid process of the basioccipital.

Above the facial notch is the superior anterior process (the ala otosphenoidea of SIEBENROCK, 1893 b: 527, processus trabeculae superior, 1895; the crista alaris of OELRICH, '56) which appears to be a dermal extension from the otic capsule. This process is well developed and laps inside the ventral cranial wall process of the parietal in *Tupinambis, Anguis,* or *Varanus.* It is only slightly developed in the Iguania (generally) and lacking in *Chamaeleo.* This dermal process is well developed in amphisbaenids and ZANGERL ('44: 424) reported a separate center of ossification in a young specimen of *Leposternon microcephalum.*

The geckos and pygopodids differ in that the anterior margin of the prootic is nearly a straight line which extends from the tip of the superior process to the posterior process of the crista prootica which lies just lateral to the outer aperture of the facial canal. A trigeminal (V) notch and a ventral anterior process of the prootic are lacking as is also a distinct posterior clinoid process of the basisphenoid.

VAN PLETZEN ("46: 58—59, Fig. 9) described a bony squame applied to the outer surface of the prootic overlying the horizontal semicircular canal, an os investiens, which may give rise to the crista prootica—a possibility suggested by WATSON ('16: 342). The crista prootica (otosphenoidal crest) is a thin flange extending outward and downward from near the ventral margin of the prootic. It usually begins as a strong projection from the ventral margin of the inferior anterior process about midway along that process. The margin of the crista extends back and slightly upward to another particularly strong extension at a point on a line with the anterior margin of the prootic above the incisure. This region is the anterior end of the crista prootica of the gecko where it is drawn down and back as a long process. Thus the geckos and pygopodids (*Xantusia* ?) appear to lack a distinct inferior anterior process of the prootic, as do the amphisbaenids. From the area of greatest development, the crest tapers to a point near the tip of the paroccipital process.

Beneath this flange is a broad channel with an arched roof, also prootic. An-

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terior to the outer exposure of the opisthotic the prootic has a ventral cranial wall extension which contacts the basisphenoid, and its outer cover of parasphenoid. The facial nerve (VII) canal opens (one foramen in most but two in *Varanus* or *Ophisaurus*) into the channel anterior to the opisthotic exposure and near the roof. This channel can be identified as the sulcus of the vena capitis lateralis (the recessus vena jugularis of OELRICH, '56; *see* Figs. 5, 7 of SÄVE-SÖDERBERGH, '47).

The superior anterior process of the prootic forms the lateral margin of the blood vessel and nerve sulcus in the amphisbaenid (Fig. 9). This flange is continued forward by the lateral margin of the parietal. Inside this flange the anterior margin of the prootic contacts the orbitosphenoid above the position of the trigeminal incisure.

The inner aspect of the prootic bears (entirely, or most of) the internal auditory meatus in which three (or four) foramina are observed. The most anteroventral of these is the facial foramen, the dorsoanterior one and the posterior one are for the auditory nerve.

In some of the iguanids there is a pointed process projecting anteriorly from the medial wall of the prootic above and anterior to the internal auditory meatus. OELRICH ('56: 16) has called this the supratrigeminal process and pointed out that it passes between the vena cerebralis media (above) and the trigeminal nerve (below) to attach to the pila antotica. This process is best developed in *Ctenosaura;* it is distinct in *Iguana* or *Sceloporus*, and slight in *Conolophus* or *Anolis*. Such a process occurs in other families; it is characteristic of agamids (SIEBENROCK, 1895: 1103; *Uromastix* has a small one), it is small in *Eumeces* or *Mabuya* (RAO and RAMASWAMI, '52, Fig. 16). There is a trace of this process in *Tupinambis* and virtually none in *Varanus, Heloderma* and *Ophisaurus*.

The prootic is usually well fused to the adjacent endocranial bones in adults (not in young) but in some genera the sutures can be partly detected (*Tupinambis*) or well marked all around (*Varanus*). The suture with the basisphenoid and the adjacent part of the opisthotic is usually distinct.

Opisthotic (Figs. 1–6, 9, 12)

The opisthotic encloses much of the inner ear. It forms the posterior medial margin of the fenestra vestibuli, encloses the posterior ventral parts of the utriculus, sacculus, and lagena, much of the crus (sinus superior of superior utriculus), the posterior vertical canal ampulla and the endolymphatic duct. The internal perilymphatic foramen (p. 7) opens between this bone and the prootic.

The opisthotic arises in the otic capsule and almost immediately fuses with the exoccipital so that the boundaries cannot be determined. There is some question in the adult which bone forms the paroccipital process (mastoid process of mammal). This process can be viewed as entirely opisthotic (as in *Sphenodon*) or as covered posteriorly by an extension of the exoccipital. The first approach is most agree-

able, the boundary of these bones is thus marked in the adult roughly by the foramina of the metotic fissure (IX, X, XI).

The opisthotic contacts the epiotic above, the prootic in front and its paroccipital process largely supports the quadrate. The paroccipital process may be thick or thin (geckos) generally it is quite broad and extends well out from the cranium proper. This process is poorly developed in burrowers in which the cranial wall is rounded and the arches lost (*Ophioseps, Feylinia, Anniella* and amphisbaenids). Reduced processes occur in many pygopodids, skinks (*Anelytropsis, Chalcides, Voeltzkowia*) and *Anguis*. Long processes are a part of large supratemporal and posttemporal fossae.

The relationship of this process to the intercalare, the supratemporal, the squamosal and quadrate is described below.

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Epiotic

The supraoccipital of the mammal arises from bilateral centers appearing in the synotic tectum or tectum posterior; it does not arise in the ear capsule or invade the ear capsule. In the lizard there are three centers in this region (*Chamaeleo*—Parker, 1880—85; *Lacerta*—Parker, 1879, Siebenrock, 1894, de Beer, '37; *Blanus*—Zangerl, '44: 421, cited von Bedriaga as observing these centers; *Calotes*—Ramaswami, '46), one on either side in the otic capsules, and a median anlage in the synotic tectum. The single median center is comparable to the pair of bilateral centers of the supraoccipital of the mammal or early amphibian; the otic capsule center, the epiotic, of either side belongs to the otic capsule. What appear to be separate epiotic centers arise in the otic capsules of some mammals, in birds, in lizards and the Crocodilia. Since a history for this bone is not known it has been assumed to be nothing more than a recurrent neomorph or nonexistent. The place of the epiotic in the osseous system is further confused by the suggestion that the tabular bone has invaded the chondrocranium (parotic process) to produce an endochondral center lateral to the supraoccipital (de Beer, '37: 144; Romer, '56: 66).

The epiotic of the lizard encloses the dorsal parts of the two vertical semicircular canals and a part of the crus as well as the terminal part of the endolymphatic duct. It is cupped around the top of the cavum capsularis (Rao and Ramaswami, '52: 259) or cavum vestibulare, the nearly undivided cavity occupied largely by the utriculus and sacculus. The fused epiotic-supraoccipital contacts the prootic, opisthotic and exoccipital; it may be fused with these bones.

The epiotic of most agamids is peculiar in that there is a fenestra below the anterior semicircular canal just before it reaches the crus (lacking in *Liolepis, Uromastix* and *Moloch*—Siebenrock, 1895: 1094). This fenestra opens through to the outside from the dorsal part of what might be viewed as the subarcuate

fossa. Such a fossa is usually lacking in lizards (*Ctenosaura, Mabuya* or *Tupinambis*) but is found in *Anolis* where in a single specimen a fenestra was found on one side.

The inner aspect of the epiotic bears the small opening for the endolymphatic duct just above the point where the suture between the prootic and opisthotic meets the ventral margin of the epiotic.

Parasphenoid (Figs. 1, 3, 4, 6, 9)

The parasphenoid has been discussed by LAKJER (1927). As a generality it can be said to be present throughout the group although it fuses early with the basisphenoid and cannot be identified as a separate element. This bone is not lacking in *Chamaeleo* (PARKER, 1880—85; 101) as there is a typical parabasal canal.

In *Lacerta agilis* (the only adequately studied form, *see* DE BEER, '37) the parasphenoid arises from three centers which can be identified as the rostroparasphenoid and basiparasphenoids (the later may represent the fused sella- and basiparasphenoid centers of birds). These three centers soon fuse and anchylose to the overlying basisphenoid, after which the development of this dermal component cannot be followed. Although only poorly developed in the lizard this bone is probably similar to that of *Sphenodon* (where only bilateral basisphenoid centers have been described but in which a rostrum develops, probably from a third center).

Generally the cultriform process (rostroparasphenoid) is present and fused to the basal plate. A rostrum appears to be lost (perhaps through secondary reduction) in some specimens of *Mabuya carinata* (RAO and RAMASWAMI, '52: 261), most chameleons, some geckos (slightly ossified or paralleled by calcification in the "presphenoid" cartilage), pygopodids (UNDERWOOD, '57: 221), some cordylids, *Anniella, Lanthanotus, Xenosaurus,* some specimens of *Eumeces laticeps,* and *Heloderma.* This process may be ossified but separate from the basal plate in some specimens of *Mabuya carinata* and *Cordylus polyzonus* (VAN PLETZEN, '46, Fig. 2). Its absence or lack of continuity with the rest of the basis cranii appears to be as much an individual variation as one specific for a group.

In amphisbaenids the parasphenoid appears to be well developed covering the broad base of the skull. The rostral part may be present in *Amphisbaena fuliginosa* in which ZANGERL ('44: 421, Fig. 2) described a median splint between the orbitosphenoids. In *Monopeltis,* KRITZINGER ('45: 183) described a strongly developed rostrum extending up between the trabeculae as a vertical plate with lateral flanges above. The lateral extensions are held between lamellae of the frontals. There are similar lateral extensions of the ventral margin forming the ventral plate of an I-beam. The ventral and dorsal plates of this beam extend further forward than the vertical plate; the dorsal plate, above the lamellae of the frontal, reaches into the nasal capsule and the ventral plate, below the inter-

orbital septum (trabecula communis), extends forward nearly to that capsule.

In *Amphisbaena alba* the parasphenoid rostrum in short and blunt, not extending beyond the vertical of the optic nerve foramina (Fig. 9). In *Trogonophis* (BELLAIRS, '40—50) it is long and thin but ends at the vertical of the optic foramen. In neither is the interorbital septum ossified (as suggested by ZANGERL, '44: 424); in *Rhineura,* where the septum does ossify (BELLAIRS, '40—50: 508), it fuses with the orbitosphenoids and does not form the rostrum.

The basioccipital process ossicles have been presumed to be the posterior bilateral parts of the parasphenoid by VAN PLETZEN ('46: 58) who said that, "... in an embryo of Cordylus in which the parasphenoid is not yet fused to the basisphenoid these plates form the postero-lateral parts of the membrane bone, thus proving that the bony squames present in the adult are posterior prolongations of the parasphenoid only." His figures do not support this statement. His figure 2 shows the "squames" in their proper position at the tip of the basioccipital processes on a common vertical with the fenestra vestibuli while the cross-section, figure 8b, shows them at the same vertical as the fontanelle between basioccipital and basisphenoid. LAKJER ('27: 82, 159) called this ossicle (the X bone of Zangerl) an epiphysis on the tuberculum sphenoccipitale, agreeing with Brühl in this (*see* under basioccipital).

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Basisphenoid (Figs. 1, 3—6, 9, 12)

The basisphenoid forms the floor of the cranium in front of the basioccipital. Laterally it extends out and down as basipterygoid processes to contact the pterygoid.

The basipterygoid processes are much reduced in amphisbaenids but still distinct in *Trogonophis* (ZANGERL, '44, Fig. 14). These processes have a cartilaginous epiphyses which is not to be confused with the pterygoid meniscus. The process epiphysis can be calcified in some lizards (*Tupinambis*) but is always closely applied to the surface of the basipterygoid process.

As pointed out by MCDOWELL and BOGERT ('54: 104) the basipterygoid process of the diploglossan (anguimorph) type has a distinct venous groove passing above it which continues the sulcus of the vena capitis lateralis forward into the orbit. In most lizards the anterior end of the sulcus forms a distinct pit behind the base of the basipterygoid process at the entrance of the carotid canal (parabasal or vidian canal). This pit was identified by McDowell and Bogert as the entocarotid fossa.

Above and behind the basipterygoid processes the basisphenoid contacts the prootic. At the anterior end of this suture there is a small posterior clinoid process (alary or alar process of RAO and RAMASWAMI, '52: 262, Fig. 10). The dorsum sellae extends between the bases of the clinoid processes of either side; the base of each process is penetrated on its inner aspect by the canal of the abducens

nerve. Posterolaterally there is a process (covered by parasphenoid and perhaps entirely parasphenoid) which extends out and down almost to the tip of the basioccipital process.

In the amphisbaenids (ZANGERL, '44: 420) there is no bony sella or dorsum sellae. There is no abducens canal; the nerves III, IV and VI are lacking. Nerve V exits through the large trigeminal foramen in the cranial wall below the superior anterior process of the prootic and above the basisphenoid (Fig. 9).

The carotid or parabasal canal penetrates the fused basiparasphenoid. Presumably it passes between the two bony elements. The canal enters the compound bone dorsolateral to the base of the basipterygoid process, at the anteroventral end of the sulcus vena capitis lateralis. The canal passes forward and slightly downward and branches into one passage which continues forward to emerge on the anterior aspect of the base of the basipterygoid process and one which passes medially to emerge in the sella turcica. The parabasal canal contains the internal carotid artery and the palatine branch of the facial nerve (the vidian nerve). In the canal the artery branches; the internal carotid enters the sella, the palatine branch continues forward with the nerve. The carotid artery does not penetrate the cranial base of the amphisbaenid as illustrated by ZANGERL ('44, Figs. 1, 17) rather this artery enters the parabasal canal above the X ossicle through a notch in the ventral margin of the foot-plate of the large stapes (Fig. 9).

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Orbitosphenoid (Figs. 1, 3, 4, 6, 9)

A small orbitosphenoid (laterosphenoid of ROMER, '56: 119, or pleurosphenoid) occurs in most lizards. The problems of naming this orbit ossification stem from a lack of real similarity in the chondrocranii of the different classes (Fig. 15) and from the misconception that there are several different bones in the orbit and that all, or some, of these can occur in a single species (*see* CAMP, '42: 32—33; BELLAIRS, '40—50: 491—492). The subject of orbital ossifications cannot be easily dismissed and should be considered before continuing.

Generally the orbit region is assumed to contain a mesethmoid, a presphenoid, an orbitosphenoid and/or a laterosphenoid. These bones are presumed to be parts of the single sphenethmoid of the primitive amphibian and reptile which may or may not extend back to enclose the optic foramen. The last three elements of the series are adjacent to the optic nerve foramen and appear to constitute centers of a primordial unit derived from the sphenethmoid. Thus there are only two ossifications in higher forms representing parts of the sphenethmoid: an anterior mesethmoid and a posterior orbitosphenoid.

Proof that several "bones" are involved in this region is based on their occurrence together in the same animal. In the lizard which lacks the mesethmoid, SIEBENROCK (1892—1895) described an orbitosphenoid and a presphenoid; PARKER (1880—85) used the terms orbitosphenoid, alisphenoid and presphenoid;

and RAMASWAMI ('46: 280) described an orbitosphenoid and pleurosphenoid. Comparisons of these accounts, and others, indicates lack of agreement among them and casts doubt on the propriety of identifying more than one orbital ossification.

A similar situation is encountered in other vertebrate groups. As a case in point, the bird (*Gallus*) is described by DE BEER ('37: 282) as having an orbitosphenoid and a pleurosphenoid in addition to the mesethmoid. WEBB ('57: 119—121) discussed the several opinions regarding ossifications in the interorbital septum. One detects some regret in his remark that the ostrich lacks the "orbitosphenoid" which is replaced by a secondary extension of the mesethmoid into this region. That secondary ossification of this area occurs cannot be doubted, but that "orbitosphenoid" centers *usually* develop here as suggested by Bütchli is scarcely credible. Such centers, should they exist (there is no acceptable evidence that they do—JOLLIE, '57, based on examination of many developmental stages of many species), must be considered as comparable to the secondary dermal ossifications forming the postorbital and zygomatic process extensions of the chicken (they would appear at about the same time).

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In the mammal the "orbitosphenoid" and "presphenoid" upon examination are found to be parts of a single circum-optic-foramen orbitosphenoid (JOLLIE, '57; MCDOWELL, '58). The mesethmoid of the mammal appears to be a secondary development which may fuse with the orbitosphenoid. The "pleurosphenoid" of the platypus (DE BEER, '37: 298) is properly a part of the basisphenoid which extends into the clinoid process. Thus the evidence from the bird or mammal supports my view. The question now becomes, is the optic foramen bone the same in all—does an orbitosphenoid develop in some and a laterosphenoid in others?

That the lizard orbitosphenoid is comparable to the mammalian bone of this name has been widely assumed, at least in terms of identifying it as the orbitosphenoid (for example OELRICH, '56). To demonstrate the homology it can be pointed out that although the mammalian chondrocranium is quite different from that of the lizard, a pila metoptica is present in placentals and attaches to the basal plate of cartilage (trabeculae) lateral to the hypophyseal fenestra. The clinoid processes of the dorsum sella are presumed to be remnants of the pila antotica. In the monotreme the pila metoptica is absent but the pila antotica is present while the marsupial lacks both of these pilae (*see* GOODRICH, '30: 269—270).

If we assume that these cartilaginous pilae are properly compared from one class to another then we can say that the orbitosphenoid of the placental mammal ossifies around the optic nerve (two or three pairs of centers) in the orbital cartilage and pila preoptica and pila metoptica (and trabecular plate). The mammal thus agrees in part with the lizard in which this bone is mainly an ossification in the pila metoptica. The comparison could be carried as far as pointing out

that the so-called pleurosphenoid of the platypus (DE BEER, '37: 298) is like that described for the lizard *Calotes* by RAMASWAMI ('46: 280—or BROCK's, '40—50, x-plate in *Acontias*).

In the bird a pila metoptica is wanting (only the base is indicated but the orbital cartilage (posterior orbital cartilage which corresponds to ROMER's, '56, Fig. 38, center of "orbital laticework") is well developed and attached to the cranial floor by a series of "pilae" which change during the developmental period. The first attachment is a pila antotica; the pila antotica spuria is a later development. At first the posterior orbital cartilage lies medial (anterior) to the trochlear nerve but with time it moves lateral to that nerve and encloses the opthalmic branch (V_1) of the trigeminal nerve by the downgrowth and subsequent attachment of the pila antotica spuria to the cranial base.

Ossification of the orbitosphenoid begins in the orbital cartilage (one or two centers in the chick above and below a fenestra). Since a pila metoptica is not involved (rather a "pila antotica spuria") it is argued that the resulting bone (the "pleurosphenoid" or "laterosphenoid") is not the homolog of that of the lizard. Where this ossification appears is a matter of definition. The initial procartilage anlage is medial to the trochlear nerve (in the position of the bone of the lizard) later is is lateral to that nerve in a position matched only by the crocodilians. Thus the developmental story does not really support the assumption that the orbitosphenoid of the bird is a different structure from that found in the lizard; rather it leads to the conclusion that this is the same bone but with changed relationships (correlated perhaps with the early development of the expanded brain case?).

In the alligator the orbitosphenoid appears in what I would prefer to call the orbital cartilage (high in the broad "pila antotica" and posterolateral to the trochlear nerve). As it increases in size it encloses the trochlear nerve and extends down through the pila antotica and pila metoptica; it also sends a process down between the ophthalmic (profundus, V_1) and maxillary (V_2) divisions of the trigeminal nerve. There is no evidence (despite the assumptions of CAMP, '42: 33; or BELLAIRS, '40—50, Fig. 5C) that more than one primordial bone is involved in this region.

The bone of the alligator is much like that of the bird yet suggestive, in its secondary invasion of the pila metoptica, of the bone of the lizard and mammal. If this seems like a rather weak extrapolation it must be remembered that the orbitosphenoid of the monotreme or marsupial is quite different from that of the placental and yet apparently homologous. In the monotreme the orbital cartilage and "preoptic" root are involved; basically only the latter in the case of the marsupial.

Arguments demonstrating lack of homology of the orbit bones of different tetrapods are based on the same types of "presumed" similarities and differences as those advanced here in support of homology. The difference lies largely in the

fact that late, secondary ossifications or calcifications, which may or may not be contiguous with primordial ossifications, are not accorded recognition here. They are treated as accidental or functional modifications of the primordial skeletal plan. From this it follows that the plasticity of the chondrocranium and the relative conservatism of the osteocranium are demonstrated. New bones do not appear and disappear as capriciously as some writers suggest. Therefore it appears that the orbitosphenoid and pleurosphenoid are the same and that there is never more than one primordial ossification in the region of the optic foramen although it may have several centers of origin—this is the orbitosphenoid. An orbitosphenoid is lacking in living amniotes only in *Sphenodon* and turtles.

The orbitosphenoid of the lizard is a crescentic flat plate curving around the posterior margin of the optic fenestra, the dorsal portion expanded somewhat. This bone may extend secondarily into the pila accessoria behind the fenestra epiotica (*Tupinambis, Ctenosaura*). A slight accessory process occurs in some iguanids (*Amblyrhynchus, Sceloporus, Anolis*) and in *Brookesia. Chameleo* has a calcified accessory process which extends nearly to the parietal.

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An ossified orbitosphenoid appears to be lacking in gekkos (Wellborn, '33), *Heloderma* (Camp, '42) and *Anniella* (Bellairs, '50). This bone is not described by Barrows and Smith ('46) for *Xenosaurus* although it is probably present.

The orbitosphenoids of *Amphisbaena alba* are greatly developed and fused at the midline; they form the ventrolateral and ventral walls of the cranium (Fig. 9). The optic nerves perforate this plate on either side midway between the midline and the lateral margin and at the vertical of the tip of the parasphenoid rostrum. The unossified trabecula communis lies below the orbitosphenoids, anterior to the parasphenoid rostrum. The parietals lap inside the margin of this plate.

According to Zangerl ('44) the orbitosphenoids of *Amphisbaena fuliginosa* do not fuse and there is a bony rod between them. The rod has been described (above) as parasphenoid.

The orbitosphenoids of *Monopeltis capensis* (Kritzinger, '45: 182) are less extensive; anteriorly they are fused, the dorsal and ventral lamellae of the frontal overlap the margins. Posteriorly they are separated by a vertical plate of parasphenoid; laterally they reach up to contact the parietals and the superior anterior process of the prootics. The optic nerve penetrates the plate of either side below and behind the tip of the prootic process.

In *Trogonophis wiegmanni* (Bellairs, '40—50) the orbitosphenoids are small, fused at the midline and penetrated near the midline by foramina for the optic nerves. The parietals (?, *see* p. 23) meet at the midline above this reduced orbitosphenoid plate, the optic nerves passing down between them. In *Rhineura* (Bellairs, '40—50: 508) the trabecula communis is ossified and fused with the orbitosphenoids.

This bone of the amphisbaenid is quite unlike that of the other lizards but its

variability suggests that it is adaptively altered in this group. It is assumed to be a pila metoptica and orbital cartilage ossification (KRITZINGER, '45: 182). Fusion of the orbitosphenoids in front of and below the optic nerve foramina is a parallelism to the condition in the mammal. The enormous trigeminal foramen suggests that the pila antotica does not develop or at least does not remain in the adult.

The orbitosphenoid of the typical lizard is frequently associated with calcifications in the orbital laticework. Such calcifications have been identified as "presphenoid", "pleurosphenoid", or "alisphenoid." In some lizards (*Iguana, Amblyrhynchus, Ctenosaura, Uromastix*) calcification of the anterior part of the "taenia medialis" occurs, in others calcification encloses the fenestra septalis (*Amblyrhynchus cristatus,* CM 30106) or at least margins this fenestra dorsally. A specimen of *Iguana iguana* (CM 35157) showed the extreme of calcification. In this specimen the interorbital septum was largely calcified along with a posteriorly projecting band paralleling the parasphenoid back to the vertical of the optic nerve. The subiculum infundibuli and hypochiasmatic cartilage (*see* SÄVE-SÖDERBERGH, '47, or OELRICH, '56, Fig. 7) were also cocalcified and almost joined to the interorbital septum. A small prong of bone extended back, from that in the root of the pila accessoria, into the taenia medialis (leading to the pila antotica). The basal part of the pila antotica, at the basisphenoid, was calcified.

In those lizards in which the orbital latice is reduced (*Uroplatus*—SIEBENROCK, 1893: 522; *Tarentola, Lepidodactylus, Stenodactylus, Saurodactylus, Ebenavia*—WELLBORN, '33; and *Anniella*) even a cartilaginous orbitosphenoid is lacking.

Calcification may occasionally lead to bone. A small ossification was observed in the interorbital septum of the mosasaur, *Plotosaurus* (CAMP, '42: 33, Fig. 15). The trabecula communis (interorbital septum) of *Rhineura* is ossified (BELLAIRS, '40—50: 508) but fused with the orbitosphenoid. The ossified trabecula communis of *Aprasia* forms the "rostrum" of the basisphenoid (UNDERWOOD, '57: 221).

Squamosal (Figs. 1, 2, 5, 9, 12)

The discussion of the temporal bones of lizards (the squamosal and supratemporal) has been summarized by CAMP ('23: 346—351), GOODRICH ('30: 347—349), VAN PLETZEN ('46: 54—56) and ROMER ('56: 115—116). Goodrich has summed it up nicely: "The outer bone, which joins the jugal, is considered by many to be the squamosal (Huxley, Boulenger, Gadow, Siebenrock, Broili, v. Huene, Broom, Jaekel, Williston and others); it is called paraquadrate by Gaupp, prosquamosal by Baur and Case, quadratojugal by Watson. The inner bone next to the parietal is called supratemporal by the majority, squamosal by Gaupp and Watson, tabular by Broom, Williston, and v. Huene." To this can be added that CAMP ('23), MCDOWELL and BOGERT ('54) and ROMER ('56) called

the outer bone the squamosal; Camp and McDowell and Bogert called the inner one the tabular while Romer called it the supratemporal. Broom ('35: 13) added that "... the views that have been expressed have been so varied that one's confidence in even the greatest comparative anatomists becomes seriously shaken. The older anatomists seem to have had no very definite views at all, and a number of them at different times held quite different opinions. Kitchen Parker for a time believed that the inner bone was the squamosal: then he came to hold that it was the outer bone. Cope also changed his view, while Baur during his short though brilliant career held three different views."

Th squamosal of the lizard cannot be identified with that of the mammal with any great conviction since the history of the lizard temporal region can only be surmised. On the assumption that the lizard is a derivative of a diapsid group (resembling *Sphenodon* in some respects—see Watson, '57), one can assume that the squamosal forms part of the temporal arch and that the quadratojugal is lacking (at least as a discrete entity).

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The lacertilian squamosal lies above the articular region of the quadrate. Along with the supratemporal, parietal, prootic and exoccipital it contributes to the support of the articular area and forms part of the temporal arch. This bone is present in most lizards but where the temporal arch has been lost it may have disappeared. It is usually thought to be lacking in geckos, pygopodids, *Anniella* and amphisbaenids (Romer, '56: 123). On the basis of the reduced squamosal of *Lanthanotus* or *Heloderma* and the relatively well developed supratemporal it can be assumed that when only one bone is present it is the supratemporal. However, in the anguids and *Eumeces* the squamosal is large, the loss of a supratemporal fossa appears to have reduced the supratemporal most.

Beddard ('05a) showed the squamosal of *Uromastix* as having a parietal wing as well as contributing to the temporal arch. A furcate shape is observed not only in some agamids, and iguanids (*Phrynosoma*) but particularly in the chameleons where it helps form a distinct posttemporal arch over the posttemporal fenestra. When the squamosal is furcate, the supratemporal bone is inconspicuous. *Lyriocephalus*, an agamid, is peculiar in that the entire ventral margin of this bone is in contact with the quadrate (Siebenrock, 1895: 1113).

Supratemporal (Figs. 1, 2, 4—6, 12)

The question of whether this is the supratemporal or the tabular cannot be resolved at the moment. I have called it the supratemporal in spite of the fact that it is not used as a part of the origin of the temporal muscle (van Pletzen, '46: 56, which in the views of Brock, '32: 529, show it to be the tabular). The relative position and relationships of this bone in the lizard and its presumed phylogenetic history support the use of supratemporal (Romer, '56: 115—116; Watson, '57).

The supratemporal is generally a small splint of bone wedged between the squamosal and the parietal. It assists in the support of the quadrate and it may even invade the intercalare cartilage of the articular region (VAN PLETZEN, '46: 56). The supratemporal (and squamosal) is lacking only in the gekkos *Lygodactylus* and *Saurodactylus* (BROCK, '32; WELLBORN, '33) and in burrowers where there has been complete loss of the temporal arch: i.e., some pygopodids (*Ophioseps*) and some amphisbaenids ("Dorsal to the point where the quadrate originates there are thin superficial bones in various stages of reduction . . ." ZANGERL '44: 425—present in *Amphisbaena alba,* LAKJER, '27, Fig. 55, but not observed by writer; *Rhineura floridana,* ZANGERL, Fig. 15). The supratemporal is much reduced and concealed by the squamosal in some chameleons (*Brookesia,* SIEBENROCK, 1893 a). In mosasaurs it grows downward and upward to such an extent that it takes part in the formation of the ear capsule (ROMER, '56: 123; *see* MCDOWELL and BOGERT, '54, Fig. 10).

When only the supratemporal occurs, as in most geckos, it is shaped much the same as the squamosal of the typical lizard with a temporal arch; that is, there is a long, pointed parietal process and a short, rounded, somewhat downwardly and outwardly bent quadrate process. It is reasonable to presume—from the fact that the supratemporal appears to be in the process of reduction throughout the lizards—that when only one bone is present it is in fact the squamosal (*see* UNDERWOOD, '57: 222, squamosal and sometimes supratemporal present in geckos). Only the long parietal process supports the contention that it is the supratemporal. This latter relationship may be the result of elimination of the temporal arch by pressing it against the cranial capsule; i.e., the single bone is the squamosal (*see* CAMP, '23: 350).

Parietal (Figs. 1, 2, 4—6, 9, 12)

The parietals are generally fused at the midline, they are separate in most geckos (*Aristelliger, Uroplatus, Gekko, Hemidactylus* but not in *Hemitheconyx* or *Coleonyx*—WELLBORN, '33; MCDOWELL and BOGERT, '54), some pygopodids (*Pygopus, Delma, Aphrasia* but not *Lialis* or *Ophioseps*) and the xantusiids (and ? some anguids—ROMER, '56: 120). Fusion of the parietals is often a species characteristic among geckos: *Phyllodactylus siamensis, Coleonyx variegatus* and *C. elegans,* and *Lepidoblepharis festae* have the parietals fused while other species of these genera have them free (NOBLE, '21).

The fused parietals are also fused to the frontals in the amphisbaenid, *Bipes biporus* (ZANGERL, '44, Fig. 9). Anteriorly the parietals usually contact the frontals along a fairly straight suture which may be slightly or highly lobate in some amphisbaenids. In *Trogonophis wiegmanni* (ZANGERL, '44, Fig. 14) a lobe of the parietal extends forward between the frontals to the premaxilla; more laterally the parietal contacts the maxilla between the frontal and prefrontal.

A parietal foramen is usually present between the parietals but this foramen may be between the frontals (chameleons, a few specimens of *Uromastix aegyptia*—EL-TOUBI, '45: 5; other agamids such as *Sitana ponticeriana, Gonyocephalus kuhlii*—SIEBENROCK, 1895: 1108, and an iguanid, *Dipsosaurus*). In most iguanids and agamids it opens on the fronto-parietal suture. In the agamid *Gonyocephalus godeffroyi* this foramen is between the parietals but just behind the fronto-parietal suture. This foramen is well back between the parietals in most skinks, anguids, some cordylids, and varanids. A parietal foramen is lacking in one agamid (*Leiolepis bellii*—SIEBENROCK, 1895: 1108), geckos, pygopodids, xantusiids, teiids, *Feylinia* (Scincidae), some lacertids (DEGEN, 1111: 30), *Lanthanotus, Heloderma,* many (ROMER, '56: 121) cordylids, *Anniella* and amphisbaenids (except *Monopeltis*—KRITZINGER, '45: 188). In agamids the parietal foramen tends to grow smaller with age and even disappear (SIEBENROCK, 1895: 1109). In *Uromastix* the foramen is exceptionally large.

Viewed dorsally the parietals usually have strongly concave lateral margins and these margins show the scar of the temporal musculature. The medial margin of the muscle fossa may be strongly raised and in some iguanids (*Iguana, Conolophus*), in *Varanus gouldii* (MERTENS, '42, pl. 21, Fig. 145) and in *Amphisbaena* these margins meet to form a medial crest. There is a very tall sagittal crest in chameleons. The lateral margin may not be incised; it meets the bones of the temporal arch along a nearly straight line to roof over the supratemporal fossa in *Xantusia, Cordylus,* some *Eumeces,* and *Anguis.*

Posterolaterally the "parietal processes" extend out over the posttemporal fossa to the head of the quadrate. Such processes are related to development of the large supratemporal fossa (and its posterior exit under the posttemporal arch through the fenestra of that name) or to the presence of a deep posterior embayment in the parietals. Parietal processes are replaced by the posterior sagittal process in *Chamaeleo.*

The posterior margin of the parietals may be nearly transverse (*Cordylus*) or deeply incised (*Anguis*). It may be incised with a median forked process (articulating with supraoccipital) in *Gerrhosaurus* or transverse with such a process (not articulating) in *Xantusia.* The posterior margin may be drawn back and upward, well away from the occipital bone and ornamented with hornlike processes (dermal bone) as in *Phrynosoma solare.*

Generally the parietal is largely free of the supraoccipital, the processus ascendens of that bone fitting into a socket in the posterior margin of the parietal. In the large iguanids and some agamids the parietal and supraoccipital are in contact, usually there is a ligamentous gap between these bones allowing some movement in the parietal-occipital joint (metakinesis). The sagittal process of the parietal sheaths the large median supraoccipital process in *Chamaeleo;* they are more loosely joined in the cordylids (*see* MCDOWELL and BOGERT, '54, Fig. 28). In *Anniella,* amphisbaenids and *Ophioseps* loss of the temporal arch and round-

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ing of the cranial wall has brought the parietal and supraoccipital largely into sutural contact. A sutural contact also occurs in the mosasaur, *Plotosaurus* (Camp, '42). The parietals of *Acontias,* a skink, are fused with the supraoccipital (Brock, 40—50: 79).

The posterior margin of the parietal is peculiar in *Ophisaurus.* There are long posteriorly directed quadrate processes which are flattened above and have a narrow marginal strip abruptly stepped down behind. As this lower step approaches the midline of the skull it curves down strongly to form a vertical process contacting (or nearly contacting) the supraoccipital just lateral to the base of the processus ascendens. A fairly similar posterior margin of the parietal is observed in *Eumeces laticeps* but here the step is somewhat overhung by a thin margin of bone creating a distinct transverse fossa for the attachment of the cervical muscles.

The parietals have slight descending cranial wall laminae which may be drawn out as a descending process to contact the epipterygoid (*Uromastix, Ophisaurus, Tupinambis*). Camp ('23: 393) pointed out that these processes are long in skinks. They extend down nearly to the facial (trigeminal) notch of the prootic in *Eumeces laticeps* (also in *E. schneideri* and *Chalcides simonyi*—Siebenrock, 1892, Tafl. XII, Figs. 5, 6) and in *Xantusia riversiana* (McDowell and Bogert, '54, Fig. 25). The cranial wall processes contact or overlap the superior anterior process of the prootic (iguanids) or lap inside that process in *Tupinambis, Uromastix* or *Varanus.* The amphisbaenids are peculiar in that the lateral ventral margin of the parietal sutures with the superior anterior process of the prootic and the orbitosphenoid thus forming a complete side wall for the cranium. Bellairs ('40—50) figured the parietals as meeting above the orbitosphenoid in *Amphisbaena* but these are probably the frontals; in *Trogonophis* it may be the parietals which meet with the optic nerves passing down between them. Descending processes are lacking in *Chamaeleo.*

Frontal (Figs. 1, 2, 4, 6, 9, 10)

The frontal bones are well developed and form the cranial roof between the orbits. They are typically fused at the midline (paired when they first ossify). They remain separate in adults of some geckos (*Pachydactylus* and *Saurodactylus*—Wellborn, '33—but not in *Hemidactylus, Aristelliger, Coleonyx*), the pygopodid *Ophioseps,* ardeosaurids, xantusiids, some scincids (*Feylinia, Scincus, Chalcides, Voeltzkowia, Eumeces* but not *Lygosoma* or *Mabuya*), *Lacerta,* varanids, *Lanthanotus, Heloderma,* many anguids (*Diploglossus, Anguis* but not *Gerrhonotus*), *Anniella,* and most amphisbaenids (not in *Amphisbaena ewerbecki*?). Fusion of the frontals is related to narrowing of the cranial roof between the orbits, although this statement does not appear to apply in some cases where secondary widening might have occurred (varanids, some iguanids).

In *Trogonophis wiegmanni* (ZANGERL, '44, Fig. 14) the frontals are overlapped by a lobe of the fused parietals which extends forward to the premaxillae; the parietal is also in contact with the maxilla between the prefrontal and frontal. Anteriorly the frontal usually contacts the nasal and the prefrontal; it may contact the maxilla (few specimens of *Uromastix aegyptia*—EL-TOUBI, '45, Fig. 1; also most amphisbaenids). The frontals extend forward to the narial margin, between the nasal and prefrontal, in a few specimens of *Uromastix aegyptia* (EL-TOUBI, '45, Fig. 1), varanids, aigialosaurids and mosasaurids. The frontals may contact the premaxilla between the nasals as in the agamids *Uromastix* and *Psammophilus,* the pygopodids *Delma* and *Pygopus,* in *Gerrhonotus imbricatus* (SIEBENROCK, 1892, Tafl. XII, Fig. 8) or *G. kingi* (MCDOWELL and BOGERT, '54, Fig. 39), *Xenosaurus, Cnemidophorus sexlineatus,* occasionally as a peculiarity in *Varanus indicus* (MERTENS, '42, Tafl. 21, Fig. 141), and in some amphisbaenids (*A. alba, Leposternon* and *Monopeltis*). This feature appears to be a variable in some species, the nasals usually meeting at the midline to cut off this contact.

The frontals margin the orbit in all except some chameleons (*Chamaeleo* not *Brookesia*), some pygopodids (*Delma, Aprasia*), *Anniella,* and *Heloderma.* In these the prefrontals and postfrontal (or postorbital in chameleons) usually meet along the orbit margin. Posteriorly the frontals contact the parietal along a distinct suture which is lost only in *Bipes biporus.* This suture line is usually simple or slightly irregular but in some amphisbaenids it is deeply lobed (*Amphisbaena, Geocalamus, Trogonophis*).

The frontals enclose the parietal foramen in chameleons, a few specimens of *Uromastix aegyptia,* in *Sitana ponticeriana* and *Gonyocephalus kuhlii* (SIEBENROCK, 1895: 1108), and in *Dipsosaurus* (an iguanid). Laterally the frontal contacts the postfrontal or, when that bone is lacking, the postorbital. The frontal may have a distinct postorbital process which extends out and forward over the orbit as in *Phrynosoma* and *Gonyocephalus* (? *Lyriocephalus*).

The frontal extends into the orbit a varying amount. In geckos, pygopodids, *Acontias, Heloderma, Anniella* and amphisbaenids, and to a lesser extent in varanids, descending orbital lamellae meet at the midline below the olfactory bulbs of the brain (interdigitating suture in *Heloderma*—fused in some amphisbaenids, but not in *A. alba, A. ewerbecki, Monopeltis, Trogonophis;* fused in *Delma* fraseri, a pygopodid). Well developed orbital lamellae occur in anguids and *Eumeces;* these may contact the palatine in *Eumeces* (also in the agamid *Lyriocephalus*).

Nasal (Figs. 1, 2, 4, 9, 10)

The nasal bones are usually not fused at the midline but they are in some geckos (*Uroplatus, Lygodactylus, Lepidodactylus, Ebenavia*), the chameleon

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Brookesia, most varanids (not *V. semirex boulengeri* or *V. caudolineatus*—MERTENS, '42: 167, 176), *Lanthanotus*, some burrowing scincids (*Feylinia* not *Anelytropsis*), *Eremias knoxii* (a lacertid—BROOM, '35, Fig. 5B), aigialosaurids and mosasaurids. The nasals do not meet at the midline in some specimens of *Cnemidophorus, Gerrhonotus, Varanus indicus* (uncommon—MERTENS, '42: 155) and some amphisbaenids (*Amphisbaena alba, Leposternon microcephalum, Monopeltis capensis, Trogonophis wiegmanni*).

The nasals are usually large and prominent but show marked reduction in chameleons, *Uromastix, Xenosaurus* and varanids. In *Chamaeleo* (not in *Brookesia*) the posterior part of the splint-like nasal is separated from the prefrontal by a fontannelle while the anterior parts of these bones are in contact. The nasal is excluded from the external narial opening by the prefrontal which anteriorly contacts the prenarial process of the premaxilla. The nasal is excluded from the narial margin by the maxilla in *Platysaurus guttatus* (Cordylidae—BROOM, '35, Fig. 5C). The nasals are laterally compressed by the posterior extension and huge size of the external narial openings in *Uromastix, Varanus*, aigialosaurs and mosasaurs. Those of the first two genera are very similar in form. A small fontanelle is found posteriorly between the nasals in *Agama* or *Moloch;* this fontanelle is plugged by the contact of the premaxilla with the frontal in *Uromastix* (EL-TOUBI, '45: 4—5).

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Premaxilla (Figs. 1—4, 9—11)

The premaxilla of either side arises separately but usually fuses with its mate as adult size is reached. Separate premaxillaries are retained in the adults of most species of the family Scincidae (unpaired in *Scincus*—SIEBENROCK, 1892: 176; EL-TOUBI, '38: 16—or *Feylinia*—CAMP, '23: 299) and in some geckos (*Phyllurus*—some geckos, iguanids and many skinks, ROMER, '56: 120). The premaxillaries are most reduced in the chameleons where they fuse very early in development (PARKER, 1880—85; BROCK, '32) and form an edentulous splint in the adult. Many agamids are intermediate toward the chameleons.

A single midline tooth is present in some species of *Agama* and *Phrynocephalus* (SIEBENROCK, 1895: 1119) and the amphisbaenid *Rhineura. Monopeltis* appears to have only a single tooth (ZANGERL, '44) but KRITZINGER ('45: 178) found four teeth on the fused premaxillae. A midline tooth along with lateral teeth is present in many lizards. In scincids the right premaxilla is generally larger than the left and has one more tooth (*see* SIEBENROCK, 1892: 177); the median tooth probably belongs to the right premaxilla. In *Uromastix aegyptia* and *Aporoscelis* the incisors (3) fuse together to form a single cutting edge (the central tooth may form the entire cutting edge). A large midline tooth is characteristic of (some) amphisbaenids (ZANGERL, '44: 425).

The premaxillae have thin splint-like prenarial processes (processus nasalis)

which are usually fused and which contact and sometimes separate the anterior ends of the nasals. The exception to such processes is in some amphisbaenids and *Aprasia* (pygopodid) where the body of the bone ends bluntly in contact with the nasals. In *Trogonophis wiegmanni* (ZANGERL, '44, Fig. 14) the truncated (fused) posterior margin of the premaxillae contacts the fused parietals between the frontals. In *Anguis* the nasal process widens suddenly just before tapering to a short dorsoposterior point. The fused prenasal splint may pass between the nasals and almost, if not, contact the frontals (*Uromastix, Delma* and *Pygopus, Gerrhonotus, Cnemidophorus, Xenosaurus, Leposternon*—ZANGERL, '44, Fig. 17, and a specimen of *Amphisbaena alba*).

The palatine portion of the premaxilla contacts the maxilla and usually the vomer. The medial palatal processes, which usually do not fuse, may be excluded from contact with the vomer by processes from the maxillae which approach or meet at the midline. These processes in many lizards bear a spine (the spina premaxillaris, incisive process or prevomerine process). The spine may be present when the processes are absent (iguanids) or be placed well in front of the processes (*Varanus*). In some iguanids (*Conolophus*—also in *Varanus*) this spine is long and pointed; in *Anolis* it is a squat knob. A short spine is present in *Gekko, Xantusia, Xenosaurus,* cordylids and lacertids. In anguids (*Ophisaurus* and *Anguis*) it is a bipartate, plate-like structure while in *Heloderma* there are twin knobs. In *Eumeces* it is a round knob which involves the anterior medial tips of the vomers. *Amphisbaena alba* has a long cartilaginous process extending down from between the anterior tips of the vomers. A bony spine is lacking (as are palatine processes) in (most) agamids, chameleons, (some) pygopodids, (some) geckos, *Tupinambis* and amphisbaenids.

To either side of the premaxillary spine, or just anterior to it are the premaxillary foramina. Such foramina are present in most lizards (distinct in large iguanids, *Varanus,* anguids); they are absent in chameleons, geckos, pygopodids, *Tupinambis,* some scincids (absent in *Eumeces* present in *Mabuya*), lacertids, cordylids and amphisbaenids. Loss of these foramina (or lack of well marked foramina) is a modification from the more primitive common pattern of lacertilians.

The alveolar bands of the premaxillae are separated partly from the maxilla by a fontanelle in *Anguis, Diploglossus, Ophisaurus, Mabuya, Cordylus* and *Tupinambis.* This fontanelle is somewhat more posterior in *Varanus.* The alveolar portion projects far posteriorly in some amphisbaenids (back to contact the ectopterygoid in *Monopeltis* and *Leposternon*) and *Lialis* (a pygopodid—MCDOWELL and BOGERT, '54, Fig. 20).

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Head Skeleton of Lizard

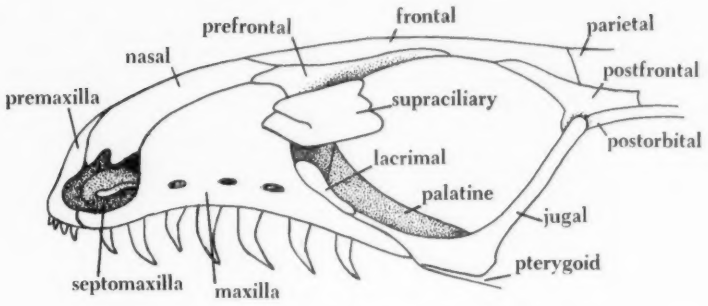

Fig. 10. Lateral view of the snout of *Anguis fragilis*.

Maxilla (Figs. 1—4, 9—11)

The maxilla is the main tooth-bearing bone of the lacertilian skull. The teeth may be acrodont (chameleons, agamids, *Trogonophis* and allied amphisbaenids—ZANGERL, '44: 425; *Trogonophis, Pachycalamus, Diplometopon, Agamodon*—GANS, '58: 441), pleurodont (iguanids, geckos, Infraorder Leptoglossa and most amphisbaenids), subpleurodont (Infraorder Diploglossa), or subthecodont, (pygopodids); they may be heterodont (agamids, the iguanid *Uraniscodon*) or homodont (most lizards).

These terms are rather meaningless, for example in *Tupinambis nigropunctatus* there are triconid incisors on the premaxillae, large conical teeth anteriorly on the maxilla, large blunt teeth in the middle and smaller triconid teeth with a main central cone at the posterior end of the tooth row (i.e., they are heterodont). The teeth in this species are anchored by a bony basal growth which is erroded at its inner base as the replacement tooth begins to form. Eventually the old tooth stands above the cavity in which the new tooth is forming. The old tooth is still attached on three sides to the sheet of bony material roofing the tooth groove of the jaw. The old tooth then drops out leaving the new tooth in a distinct socket. The question now is what does pleurodont or subthecodont mean in this case? (*see* ROMER, '56: 442).

Another detail of tooth structure and replacement enters here. BOULENGER (1885, II: 330) said the teeth of *Tupinambis* (teiids) are not hollow at the base as compared with lacertids. However, this is really a quantitative difference: a small hollow versus a larger one. The arrangement of teeth in replacement has been commented on by MCDOWELL and BOGERT ('54: 102) and GANS ('57) but there is much yet to be learned.

The maxilla sutures with the premaxilla below the nasal aperture and on the alveolar surface. It may have anterior palatal processes which meet or nearly meet at the midline. There is a median fontanelle between these processes and the anterior ends of the vomers in *Uroplatus* and *Gekko verticillatus*. The anterior

palatal processes meet at the midline in most agamids (not in *Moloch*), many iguanids (*Sceloporus*, not *Conolophus* or *Anolis*), chameleons, *Xenosaurus*, *Cordylus*, and *Tupinambis* (not in *Cnemidophorus*). Such processes are present in other genera (*Shinisaurus*, anguids) but do not cut off the vomers from contact with the premaxillae. Anterior palatal processes are absent in *Anolis* (the maxillae meet at a point above the anterior ends of the vomers), *Xantusia*, pygopodids, and *Heloderma*.

Along the alveolar length of the maxilla there is a slight palatal extension; midway there is a greater projection, the posterior palatine process. This process may be well marked (*Sceloporus*, *Eumecis*) or scarcely differentiated (*Tupinambis*, *Anguis*—Fig. 11); it is the region of contact with the palatine bone. Posterior to this process the maxilla contacts the ectopterygoid along its medial and posterior margins, as well as the jugal posteriorly and dorsally. In the orbit anteriorly, below the lacrimal canal, and dorsally on the external surface of the snout the maxilla contacts the prefrontal.

The lacrimal usually forms the orbit margin between jugal and prefrontal (*Tupinambis*); in anguids (*Ophisaurus*, *Anguis*) the maxilla forms the depressed orbit margin between the prefrontal and lacrimal but it is partly overlapped by the palpebral. Dorsally on the snout the maxilla contacts the nasal and anteriorly forms the posterior margin of the narial aperture. It may not contact the nasal dorsally, touching only the prefrontal which forms part of the nasal margin (chameleons, *Uromastix*, *Phrynosoma*). It may contact the frontal here (few specimens of *Uromastix*—El-Toubi, '45, Fig. 1; most species of *Amphisbaena*, *Rhineura*, *Bipes*, *Geocalamus*, *Trogonophis*—Zangerl, '44). In the cordylid *Platysaurus guttatus* (Broom, '35, Fig. 5C) the maxilla contacts the premaxilla above the external naris thus excluding the nasal from the narial margin.

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Prefrontal (Figs. 1, 2, 4, 9, 10)

The prefrontals are always present and usually well developed. There is usually a distinct frontal process which may extend back to contact the postfrontal (see above). There is an anterior orbital process that extends down medial to the lacrimal duct. In some lizards this bone is presumed to be fused with the lacrimal.

The lateral margin of the prefrontal is notched for the lacrimal duct. When the lacrimal bone is missing the prefrontal may largely enclose this canal (*Pygopus*, a ventral opening is closed by the maxilla).

The prefrontal has a strong supraorbital process in *Phrynosoma*, *Corytophanes* and *Lyriocephalus* which may connect with a forward directed process from the postorbital process of the frontal to form a supraorbital arch enclosing a supraorbital fontanelle.

The prefrontal of *Trogonophis wiegmanni* (Zangerl, '44, Fig. 14), an amphisbaenid, is much reduced in size and isolated from the frontal by a splint of the

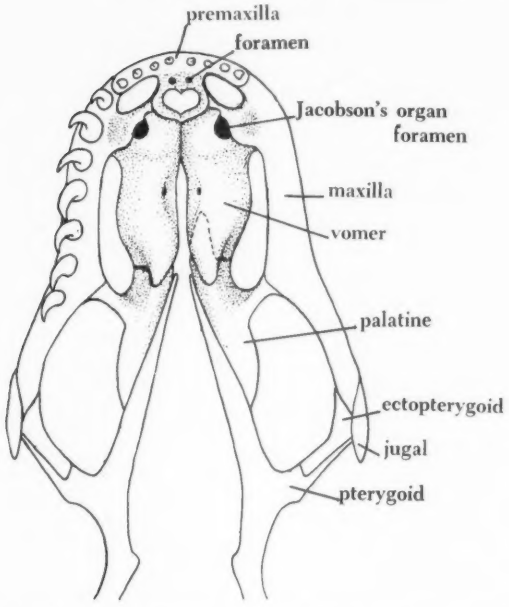

Fig. 11. Ventral view of the anterior part of the palate of *Anguis fragilis*. Dashed line indicates the process of the palatine above the vomer.

maxilla and parietal. In *Rhineura* and a specimen of *Amphisbaena alba* the prefrontal contacts the parietal. In *Monopeltis* (KRITZINGER, '45: 180, 183) the prefrontal is very small and does not margin the orbit: it does lie medial to the lacrimal duct as in other lizards.

Supraciliary or Palpebral (Fig. 10)

Generally the prefrontal is associated with a supraciliary bone which projects back over the eyeball. The supraciliary appears early in the development of *Varanus* (DERANIYAGALA, '58) being present at 78 days of development (in the egg) when the other cranial bones are just beginning to form. A palpebral is known to occur in most scincids (exceptions?), gerrhosaurids (not *Cordylus*—VAN PLETZEN, '46: 54), many anguids (*Anguis, Gerrhonotus, Ophisaurus*) and lacertids (*see* SIEBENROCK, 1892: 182—185; 1894: 82), also in *Shinisaurus, Xenosaurus, Varanus,* some mosasaurs (*Platecarpus* but not *Plotosaurus*—CAMP '42: 32), and *Anniella.*

BROOM ('35: 16) reports a supraciliary in some species of *Ameiva* (a teiid) but a check of five species (*ameiva, thoracica, dorsalis, exul, atrigularis*) revealed only a triangular pad of dense connective tissue here (even this is lacking in *Tupinambis*). ROMER ('56: 121) said a supraciliary is found in some geckos. The

palpebral figured by McDowell and Bogert ('54, Fig. 23) for *Aristelliger* does not compare with that of other lizards (it might be viewed as a remnant of the postfrontal).

In the skinks, lacertids and anguids the palpebral underlies the supraorbital osteoderms which sheath the head. It it only lightly attached to the prefrontal by connective tissue and easily detached along with the osteoderms. Broom ('35: 16) suggested that the varanid supraorbital bone is in fact a specialized osteoderm. It is doubtful that the varanid supraciliary can be distinguished from that of other lizards. Generally osteoderms are lacking in this group but when they are present (McDowell and Bogert, '54, plate 12) they lie external to the level of this bone (no osteoderms occur in the skin over the orbits). The early origin of the supraciliary in *Varanus* (Deraniyagala, '58) argues against its being an osteoderm. Siebenrock (1894: 251) stated that the palpebral is preformed in cartilage but I doubt that this course needs to be followed to assume homology.

Phrynosoma, Corytophanes and *Lyriocephalus* are peculiar in that a process of the prefrontal appears to replace the supraciliary. A bony supraciliary is also lacking in *Lanthanotus, Heloderma,* all Iguania (exceptions?), *Cordylus,* teiids and amphisbaenids.

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Lacrimal (Figs. 1, 2, 4, 10)

A small free lacrimal is usually present but none is evident in some lizards. Loss of this bone has been attributed in some instances to fusion with the prefrontal (partly fused in some *Eumeces*—Kingman, '32: 284, but still recognizable; fused to prefrontal in *Xantusia riversiana*—McDowell and Bogert, '54: 94). Generally this bone appears to be lost, not fused (as in *Microsaura*—Brock, '40: 231). The lacrimal is lacking in some iguanids (*Phrynosoma*), some agamids (Siebenrock, 1895: 1126, present or absent within a genus—*Calotes, Agama*), some chameleons (*Brookesia, Microsaura,* present in *Chamaeleo*), in geckos and pygopodids (Wellborn, '33; McDowell and Bogert, '54: 92), *Xantusia vigilis* (Camp, '23: 362), some scincids (*Tiliqua, Acontias, Egernia, Feylinia, Voeltzkowia*), some cordylids (*Chamaesaura* but present in *Platysaurus, Gerrhosaurus, Cordylus*—van Pletzen, '46: 52), and in amphisbaenids. Camp ('23: 362) is in error in stating that this bone is lacking in the Scincidae (including *Feylinia*), "Gerrhosauridae", Lacertidae and "Zonuridae." It is probably this statement that prompted Romer ('56: 121) to state its absence in "most leptoglossans."

Camp ('23: 362) has identified the bone forming the posterior margin of the lacrimal duct in geckos as the lacrimal (identified as such in *Lygodactylus* by Brock, '32 525) whereas others identify it as the jugal. Although it is described as the jugal in this account, this is done only on the assumption that the lacrimal dissappears first in lizards. Reduction of the lacrimal appears to be accompanied by extension of the jugal into its area.

The lacrimal forms the outer margin of the lacrimal duct in most lizards, the medial margin being prefrontal. The lacrimal may be penetrated by the lacrimal duct according to CAMP ('23: 362) but I know of no case of this. When the lacrimal is missing the maxilla usually takes its place (*Uromastix,* the scincid *Tiliqua*) or the jugal may do this (*Egernia*). In *Gekko gekko* this opening is between the prefrontal and jugal while in other geckos it may be between prefrontal and maxilla or all three bones may be involved (WELLBORN, '33: 183). The lacrimal canal is usually single but in *Varanus* and *Lanthanotus* it is double. In the former the more ventral opening is entirely enclosed by the lacrimal; the lacrimal extends medially between the openings in the latter (MCDOWELL and BOGERT, '54: 48). This opening is very large in some agamids.

Viewed laterally the lacrimal is usually an elongated splint margining the orbit between prefrontal and jugal. In some iguanids (the large species and *Sceloporus*) it is much shortened but deeper, quite square in outline; it is a reduced but long splint in *Anolis.* In *Varanus* and *Heloderma* this bone appears to be somewhat shortened and swollen (CAMP, '23: 362).

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Jugal (Figs. 1—4, 10—12)

The jugal forms the ventral and posterior margins of the orbit. It is almost always present being missing only in (some geckos?—present in *Lygodactylus* and all others described) some pygopodids (*Delma* and *Ophioseps*) and most amphisbaenids (ZANGERL, '44, did not observe this bone but described an "ectopterygoid" here in *A. ewerbecki* while LAKJER, '27, Figs. 55—59, illustrated it in *Amphisbaena alba, Trogonophis wiegmanni* and *Leposternon microcephalum;* my own dissection of *A. alba* failed to reveal it).

The general loss of this bone in amphisbaenids is paralleled by its reduction in other burrowing forms (*Feylinia* and *Anniella*). Reduction (or loss) accompanies loss of the orbital and temporal arches. Loss of these arches occurs also in the geckos and pygopodids where the jugal is a small vestige associated with the posterior alveolar angle of the maxilla; this vestige may lie entirely within the orbit behind the level of the lacrimal foramen. Reduction of the jugal begins with loss of contact with the postfrontal and or the postorbital as in *Varanus* or *Lanthanotus.* In the latter the temporal arch has already been lost.

The jugal is usually large and well developed; it is exceptionally so in agamids. Anteriorly it usually abuts against the lacrimal and maxilla (externally) and the ectopterygoid (internally), the postfrontal and/or postorbital posterodorsally. In the typical members of the Scincidae it does not contact the lacrimal anteriorly. Posterior contact may be with both postfrontal and postorbital (primarily the former in *Chalcides, Gerrhonotus, Anguis, Ophisaurus, Anniella*) or with the postorbital only (Iguania, *Lygosoma,* cordylids). In most of the Iguania and in *Xenosaurus* the jugal contacts the squamosal.

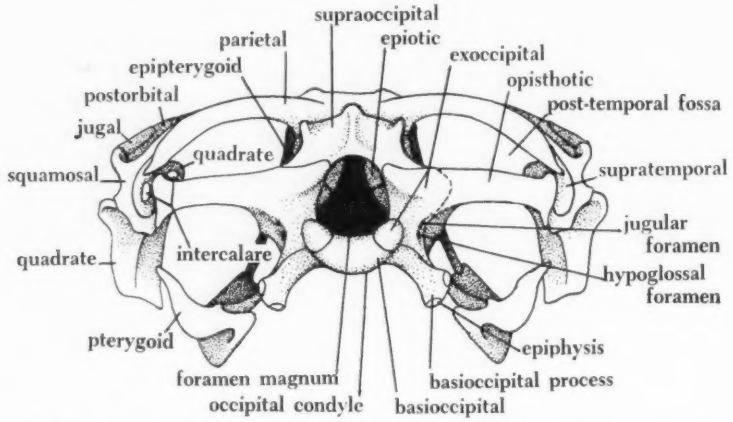

Fig. 12. Posterior view of the skull of *Uromastix aegyptia*. The dashed line indicates the presumed boundary of the exoccipital and the opisthotic bones.

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"*Quadratojugal*" and *Intercalare* (Fig. 12)

A quadratojugal has been described for lizards by several authors. The quadratojugal of Watson is the squamosal (*see above*). KINGSLEY ('26, Fig. 107) labeled the postorbital as the quadratojugal but in the text indicated that this bone was lacking in the lizard. REESE ('23) described the postorbital of *Tupinambis* as the quadratojugal. BROCK ('32: 523) described a bony splint on the outer surface of the quadrate of *Lygodactylus* as this bone; a thickened mass of tissue was observed in this region in *Mabuya*, however, RAO and RAMASWAMI ('52: 246) did not observe it in the species they studied. This bone has not been observed in prepared gecko skeletons nor has it been reported in other studies of development.

BROOM ('25) described a bony ossicle in *Tiliqua scinoides* lying above the quadrate and external to the squamosal. A similar ossicle was observed by KINGMAN ('32) in *Eumeces schneiderii*. The latter (p. 282) described it as a small, oval, dermal bone covering the aperture in the top of the quadrate and (p. 287) speculated that this might be the remains of the "quadrate-jugal."

An ossicle, not closing the foramen or notch of the quadrate, was observed in *Iguana, Ctenosaura, Conolophus, Uromastix* and *Tupinambis* and is presumably the bone described by SIEBENROCK (1892: 176) as an "epiphysis of the quadrate" in anguids and gerrhosaurids. SIEBENROCK (1894) homologized this epiphysis with the "pteroticum" of Huxley, the "adoccipital" of von Brühl and the "interarticulaire" of Laurillard. At this point there may be confusion with the intercalare, a cartilaginous mass wedged between the head of the quadrate, the paroccipital process and the supratemporal.

The intercalare is described and figured by VAN PLETZEN ('46) for *Cordylus*.

It appears to be related primarily to the extracolumella and to the paroccipital process, secondarily to the supratemporal. It has no epiphyseal relationship to the quadrate. These are the same relationships of the intercalare of *Varanus* or of other lizards. BEDDARD ('05a) identified a small ossicle in the quadrate articular region of *Uromastix* and *Heloderma* as the supratemporal 2. According to CAMP ('23: 348) Versluy's pointed out that Beddard's supratemporal 2 (also Reese's supratemporal 2 ?) was in fact a cartilaginous epiphysis on the tip of the paroccipital process formed from columellar cartilage which acted as a point of attachment for the hyoid apparatus (*see* GAUPP, '00; GOODRICH, '30, Fig. 495 C). This paroccipital epiphysis was described for several agamids (including *Uromastix*), iguanids, cordylids, *Tupinambis, Heloderma, Tiliqua* (a scincid), and *Varanus*. It was not observed in *Gekko, Lygodactylus, Pachydoctylus, Mabuya, Lygosoma, Ophisaurus,* and *Anguis*. Versluy's paroccipital epiphysis appears to be the intercalare and Leydig's paroccipital bone appears to be an ossification (or calcification) in the intercalare (*see* Figs. 103 and 109 of CAMP, '23).

Although the typical intercalare ossification lies along the outer tip of the paroccipital process a second small ossicle lay above this process in a specimen of *Uromastix,* just inside the tip of the quadrate process of the parietal (Fig. 12). This ossicle appears to be but a part of the intercalare (as suggested by Camp's figures).

Since in *Tupinambis, Iguana, Ctenosaura,* or *Uromastix* both an intercalare (bone-like and cartilaginous) and a quadrate epiphysis are present it follows that these are separate and distinct entities. MCDOWELL (in correspondence) has identified the quadrate epiphysis as "Lönnberg's ossicle" and has suggested that it is indeed a remnant of the quadratojugal. I cannot agree with this view but must go along with Siebenrock in considering it an epiphysis, of which there are many in the lizard skull.

Another type of quadratojugal has been figured by EL-TOUBI ('38, Fig. 6) for *Scincus*. This is a small ossicle, found in one specimen only, attached to the lower end of the jugal behind the maxilla and pointed towards the distal end of the quadrate. There is a heavy ligament between the angle of the jugal and the quadrate in which an ossification might occur. VERSLUYS ('36, Fig. 600) illustrated a very long splint here in a specimen of *Chamaeleo* but does not mention it in the text. Although a quadratojugal has been described by several writers and the account of Brock is quite convincing, the conclusion is that this group completely lacks the bone.

Postfrontal (Figs. 1—4, 10)

The postfrontal forms the posterodorsal margin of the orbit. It contacts the frontal (and parietal) medially, the postorbital posteriorly and ventrally and it may contact the jugal ventrally. Throughout the Iguania it tends to be reduced

in size or lacking. When missing it is largely replaced in position by the postorbital.

The problem of defining the postfrontal and postorbital when only one element is present has been discussed by CAMP ('23: 360). Although developmental information is generally lacking, what appears to be progressive reduction of one or the other element within a family can be used in the identification of a surviving unit. For example Siebenrock showed that within the Scincidae the postorbital tends to decrease in relative size until in some species only the postfrontal remains (in *Eumeces* both elements are usually well developed—SIEBENROCK, 1892: 180, said the postorbital is missing in this genus—while in *Mabuya* the postorbital is very small; in some species of *Lygosoma* and in *Egernia, Tiliqua* and *Scincus* the postorbital is lacking).

Where only one arch element occurs it has been identified as follows: 1) postfrontal—geckos, pygopods (neither in *Ophioseps*), some scincids, *Heloderma, Lanthanotus;* 2) Postorbital—a few iguanids (*Crotophytus*), most agamids, chameleons, some cordylids (ROMER, '56: 123); 3) fused postfronto-postorbital—*Xantusia* (both present in *Lepidophyma*), some species of lacertids (SIEBENROCK, 1894: 240), *Cnemidophorus* and *Teius* (teiids), most varanids (*see* DERANIYAGALA, '58—separate in adult of *V. semiremex boulengeri,* MERTENS, '42: 132), xenosaurids, and mosasaurids. Both elements are absent in the gecko *Lygodactylus* (BROCK, '32: 522), in the pygopodid *Ophioseps,* and in amphisbaenids (postorbital reported by Williston in *A. alba* but not observed by LAKJER, '27, or the writer; not observed by ZANGERL, '44, in other amphisbaenids). Both bones are present in *Ophisaurus,* not absent as indicated by ROMER ('56: 123). MCDOWELL and BOGERT ('54: 12) suggested that the postorbital of *Lanthanotus* is fused with the jugal, thus removing it as a separate entity.

Postorbital (Figs. 1—5, 10, 12)

Generally (and as a primitive feature) the postorbital and postfrontal are well developed and contribute to the orbit rim, the postorbital below the postfrontal. It is the latter which primitively contacts the jugal. The postorbital has given up its orbit margin relationship in many lizards and the jugal has come in contact with the postfrontal (anguids). This bone is missing in many lizards (see preceeding account) or is fused with the postfrontal. It is probably never fused with the jugal. Both the postfrontal and postorbital are present in *Anniella,* the latter is much reduced and poorly attached to the skull with the result it is usually lost in preparations (MCDOWELL and BOGERT, '54: 123, Fig. 42).

The postorbital along with the squamosal usually forms the temporal arch. This arch is lost in some burrowers or snake-like lizards (amphisbaenids and pygopodids) and in some lizards which might otherwise be viewed as typical (*Heloderma, Lanthanotus* and geckos). The supratemporal fossa may be roofed

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over by the postorbital, postfrontal and squamosal as in some scincids (all stages of closure of this fontanelle observed in species of *Eumeces*—no fontanelle in *E. chinensis, E. tetragrammus, E. septentrionalis;* small fontanelle in *E. laticeps;* larger in *E. obsoletus;* good sized in *E. schneiderii*); by the postorbital and squamosal in cordylids (*Chamaesaura* and *Cordylus polyzonus* have small fontanelle; *Cordylus vittifer, Platysaurus* and *Gerrhosaurus* have no fontanelle); or by the fused postfronto-postorbital and squamosal in *Xantusia.* ROMER ('56: 122) pointed out that those forms with a roofed supratemporal fossa have the head covered with a shield of osteoderms which eliminates the functional need for this fontanelle (release area for muscle bulge).

The supratemporal fossae are exceptionally large in most Iguania and reduction in size of these openings is considered an advanced or modified feature. Scincids and anguids with roofed fossae are burrowers or snake-like and show lateral compression of this roof (*Voeltzkowia—Ophisaurus, Anguis*).

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Quadrate (Figs. 1–5, 9, 12)

The quadrate is a large unit articulating above with the brain case and below with the mandible. It is capable of some forward and backward movement, i.e., streptostylic. This movement is transmitted to the snout region through the pterygoid-palatine system. The dorsal articulation is with the paroccipital process of the opisthotic bone, and the posttemporal and temporal arch units (parietal, supratemporal and squamosal). The arch units are also braced to the paroccipital process; the intercalare lies between the process and the arch units. A synovial cavity (*Cordylus,* VAN PLETZEN, '46, Fig. 6) may lie between the squamosal and the intercalare.

The quadrate may be relatively straight (some geckos, chameleons, *Xenosaurus, Heloderma, Varanus, Anniella* and amphisbaenids), or it may be arched with a concave posterior margin (slightly in *Lanthanotus,* pygopodids, some geckos and anguids; marked in *Tupinambis*). Arching of the quadrate appears to be related to development of a raised lateral margin which supports the rounded tympanic membrane (a tympanic membrane is absent in many lizards: examples include the iguanid *Holbrookia,* chameleons, some pygopodids—*Aprasia, Ophioseps, Ophidiocephalus*—UNDERWOOD, '57: 208, *Lanthanotus, Anniella* and amphisbaenids). That this correlation is not exact is shown by the large lateral conch of *Gekko gekko* which has a straight quadrate. A concave posterior margin to the quadrate is most extreme in the mosasaurid (or some turtles) where the columella may be enclosed by it.

The proximal end of the quadrate is quite massive although much of it is not involved in the articulation. There may be a notch between the articular region and the more anterior and lateral tympanic margin (*Ctenosaura, Iguana, Uromastix, Chamaeleo,* some skinks, gerrhosaurids and anguids—no noticeable margin

in *Varanus, Lanthanotus, Heloderma,* therefore no notch). The notch may be cut off by a cartilaginous strip to form a membrane closed fenestra (*Mabuya*) or this cartilaginous region may be ossified or calcified to form an epiphyseal bone which may extend well forward along the top of the quadrate (*Tupinambis* may have notch or foramen). A foramen in the top of the quadrate, with or without a distinct epiphysis, was observed in *Phrynosoma, Sceloporus, Anolis, Chamaeleo,* several species of *Eumeces* (*E. schneideri, E. faciatus, E. septentrionalis, E. brevilineatus, E. laticeps*—KINGMAN, '32: 282), *Tupinambis, Cnemidophorus, Amphisbaena* (*alba*), and *Rhineura* (ZANGERL, '44: 443). The tip of the squamosal may extend into this notch or hole in *Uromastix, Agama stellio* (LAKJER, '27, Fig. 9), and *Cnemidophorus.*

The distal end of the quadrate is considerably smaller than the proximal end. Medially it attaches to the pterygoid (syndesmosis) through connective tissue; distally it articulates with the mandible in a typical diarthrosis. The distal articular surface may appear to be double (*Gekko*) or slightly saddle-shaped with a lateral epicondyle (*Tupinambis*).

The quadrate of the amphisbaenid is firmly bound to the cranial wall throughout its length as is the pterygoid. These bindings must greatly restrict any streptostylic action.

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Pterygoid (Figs. 1–6, 9, 10, 12)

The pterygoid (perhaps only the posteropterygoid) is a Y-shaped bone. The anterior and inner process contacts the palatine, the anterior and outer process fits into the ectopterygoid or, when that bone is reduced, contacts the maxilla as well (some geckos, some amphisbaenids—*Leposternon, Monopeltis*); the posterior process extends back to the quadrate.

The palatine contact is variable but generally involves a medial process which extends forward an the medial aspect or underside of the palatine and a broad lateral process which laps the upperside of the palatine. The anterior medial process of *Uromastix* reaches almost to the posterior margin of the vomer, a situation which approaches that observed in *Sphenodon.* The near approach of these bones has been remarked by BEDDARD ('05a), SAKSENA ('42) and EL-TOUBI ('45). The situation in *Uromastix aegyptia* is the extreme for the genus and indeed for the family. It is not unlike that observed in *Anguis fragilis* (CM 28643), *Shinisaurus* or even *Heloderma.* Failure of the lizard pterygoid to contact (and overlap) the vomer is explained by LAKJER ('27) as due to the fact that the anterior end of the pterygoid, the hemipterygoid, ossifies separately and fuses to the palatine at an early stage.

The ectopterygoid process is fairly thick and blunt and fits into a notch between a ventroanterior and a dorsal process of the ectopterygoid bone. This outer process of the pterygoid contributes to the coronoid (ventral palatine) process

which articulates with the coronoid process of the mandible. An epiphyseal bone (or calcification) may be present on the outer aspect of the process (*Phrynosoma* or *Eumeces,* none in *Tupinambis*).

The quadrate process extends back and outward to end in a joint which lies in contact with the medial aspect of the quadrate. Contact with the quadrate is not always achieved. In chameleons and *Uromastix hardwicki* (Saksena, '42: 112) the pterygoid is connected by a ligament with the quadrate. In *Uromastix acanthinurus* the tip of the pterygoid reaches the quadrate while in *U. aegyptia* there is slight overlap. The quadrate process is frequently grooved on its inner aspect (*Tupinambis*) but it may be reduced to a splint as in *Agama,* much thickened and partly wrapped around the quadrate as in amphisbaendis, or laterally compressed and plate-like as in *Phrynosoma* and *Chamaeleo.*

In the middle of the pterygoid there is a medial articular area for the basipterygoid process of the basisphenoid bone and above this a dorsal cavity into which the epipterygoid fits. The articulation with the basipterygoid process is a synovial one (not in most amphisbaenids but described as such in *Monopeltis*—Kritzinger, '45: 184—; not in chameleons) and there is a cartilaginous pad on the articular area of the pterygoid.

This cartilaginous pad, the meniscus pterygoideus or the mesopterygoid of Parker, is sometimes calcified or ossified. This pad is presumed to be a part of the pterygoquadrate cartilage (*see* Rao and Ramaswami, '52: 268—269). A bony ossicle appears in this pad in some iguanids (*Conolophus, Iguana, Ctenosaura, Amblyrhynchus, Phrynosoma, Sceloporus*), in *Pachydactylus* (Brock, '32: 515, Fig. 5 A), and in *Ophisaurus.* Basipterygoid processes are poorly developed in most amphisbaenids, the pterygoid being bound to the basis cranii along much of its length.

The pterygoids of chameleons are peculiar in the broad plate-like form of the coronoid (palatine) and quadrate processes, the latter ends well in front of the quadrate. There is no epipterygoid socket.

Teeth are frequently found fused to the pterygoid. Camp ('23: 365) summarized their occurrence. Additions to his list result in the following: present in many iguanids, some scincids (*Scincus, Eumeces*), gerrhosaurids, "primitive" lacertids, aigialosaurids, mosasaurids, fossil varanids (Saniwinae), *Heloderma, Lanthanotus,* some anguids (*Gerrhonotus* and *Ophisaurus*), and some teiids (*Cnemidophorus*). The palate is toothless in some iguanids (*Phrynosoma, Sceloporus, Conolophus, Anolis*), the agamids, chameleons, geckos, pygopodids, xantusiids, xenosaurids, *Cordylus* (the Cordylinae), some scincids (*Tiliqua, Egernia*), dibamids, some lacertids (*Ophisops*), some teiids (*Tupinambis*), most anguids (*Diploglossus, Ophiodes,* some species of *Gerrhonotus, Anguis*), *Anniella,* and amphisbaenids. Pterygoid teeth are present in some species of *Crotophytus,* absent in another; usually present in *Anolis* but absent in some species—whether these teeth fuse to the underlying bone or not is something more than presence or

absence of palatine teeth. The number of teeth fused to the bone varies from one or two to distinct rows of many teeth.

Epipterygoid (Figs. 1—6, 12)

The epipterygoid (or columella cranii) is generally a well developed rod extending from a socket on the dorsal aspect of the pterygoid (fossa pterygoidei), just anterior to the centerline of the basipterygoid process, to a point of contact with the superior anterior process of the prootic (*Gekko, Varanus, Shinisaurus, Ophisaurus,* some lacertides--SIEBENROCK, 1894: 255), the adjacent descending process of the parietal (*Iguana, Uromastix, Agama, Scincus, Mabuya, Anniella*) or to both prootic and parietal processes (*Eumeces, Scincus, Tupinambis, Lanthanotus, Heloderma*).

The dorsal end, in the adult, may be capped with cartilage and enclosed in a connective tissue or ligamentous mass (*Mabuya, Agama, Cordylus*) which fixes it to the cranial wall. In the development of the gecko *Lygodactylus* (BROCK, '32, Fig 2) the cartilaginous epipterygoid is fused by its tip to the anterior wall of the otic capsule. In other lizards the epipterygoid remains free at some distance from the otic capsule.

The ventral end of the epipterygoid has a cartilaginous epiphysis which fits into a fossa in the pterygoid. This epiphysis may be continuous with a thread of tissue which is a remnant of the palatoquadrate cartilage; i.e., the epipterygoid is an ossification in the processus ascendens of the palatoquadrate cartilage (*see* VAN PLETZEN, '46: 56, 58).

An epipterygoid is lacking in chameleons, Dibamidae (BOULENGER, 1887: 434—435), and most amphisbaenids (known only in *Trogonophis,* BELLAIRS, '50: 899). The epipterygoid is very short in *Phrynosoma;* it reaches the anterior margin of the prootic just above the trigeminal notch. It is very short in some of the agamids (*Agama, Lyriocephalus* and *Calotes*—RAMASWAMI, '46, Fig. 16). The epipterygoid is short but reaches the parietal in *Ophioseps* (pygopodid) and *Anniella* because of the swollen cranium.

Generally speaking this bone is one of the more stable features of the lizard skull differing somewhat in thickness and in its dorsal attachment. The latter is sometimes difficult to determine in prepared skeletons since drying of this kinetic skull produces minor distortions.

Ectopterygoid (Figs. 1—4, 9, 11)

The ectopterygoid extends from the tip of the lateral process of the pterygoid forward and laterally as the outer margin of the palatal vacuity to contact the maxilla. The palatine anteriorly and the jugal posteriorly (within the orbit). Medially the ectopterygoid has dorsal and ventral processes which enclose the

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wedge-shaped tip of the lateral process of the pterygoid. The region of contact may be somewhat swollen with its posterolateral aspect flattened as the coronoid facet. This region may be drawn down into a well marked coronoid process (palatal process) in *Uromastix, Iguana, Ctenosaura, Conolophus, Amblyrhynchus, Sceloporus, Chamaeleo, Xenosaurus, Shinisaurus* and *Cordylus.*

In amphisbaenids (LAKJER, '27; ZANGERL, '44) and the dibamids the palatine, ectopterygoid and pterygoid are suturally united; i.e., without a palatine, or suborbital, vacuity (also called palatomaxillary fenestra) between them. Among amphisbaenids there is considerable variation in the role played by this bone. *Amphisbaena fuliginosa, A. alba, Trogonophis* or *Rhineura* have a rather typical ectopterygoid situation but lack the palatal vacuity. In *A. ewerbecki* the ectopterygoid (perhaps the jugal, *see* p. 31) does not enter into the palate at all, being a small flake of bone in the orbit (ZANGERL, '44: 430). In *Monopeltis* or *Leposternon* it is an elongate bone underlying the posterior process of the maxilla nearly or quite contacting the premaxilla anteriorly and the pterygoid posteriorly.

The palatine fontanelle is very small in *Phrynosoma coronatum,* some geckos, and *Xantusia;* in most lizards it is quite large. The bones bordering this vacuity show many variations in relationship. For example, among the agamids the pterygoid forms a part of the margin of this fontanelle except in *Uromastix* (SIEBENROCK, 1895: 1133). In the Iguania, the geckos *Rhoptropus* and *Ptenopus* (WELLBORN, '33: 185), *Shinisaurus, Xenosaurus, Ophioseps* (?), the cordylids, scincids (*Mabuya, Eumeces*) and anguids (*Gerrhonotus, Anguis, Ophisaurus*) the maxilla forms a part of the outer rim of the fontanelle.

Palatine (Figs. 1—5, 9—11)

The palatines are, according to LAKJER ('27), compound bones formed by fusion of the palatine and the anterior part of the pterygoid (anteropterygoid or hemipterygoid). JOLLIE ('57) has described the same condition for birds. It is of interest that in primitive amphibians and reptiles (including *Sphenodon* and alligator) the pterygoids contact the vomers between the palatines; this is not the case in the lizard or snake. In the young lizard, according to Lakjer, a separate ossification center arises medial to the palatine and anterior to the pterygoid; this later fuses with the palatine. This anteropterygoid was presumed to be a secondary derivative of the pterygoid (p. 72) and was described for most families of lizards. Evidence supporting the views of Lakjer is lacking from other sources.

The palatine contacts the vomer anteriorly, the maxilla laterally and the pterygoid or pterygoid and ectopterygoid posteriorly. The vomer is usually overlapped (above) by a long thin process, or this process may lie somewhat medial to it. In some lizards (*Iguana*) there is a broad overlapping process with a digitate margin.

The anterior margin of the palatine is arched upward as the wall of the

internal naris lateral to its contact with the vomer. This narial elevation may contact the descending process of the frontal (*Eumeces*). Laterally the palatine contacts the maxilla behind its posterior palatine process. The palatine may be pierced (near the maxilla) by the infraorbital canal (maxillo-palatine foramen) as in *Varanus, Anguis,* and *Ophisaurus* (*Heloderma* shows a suture dorsolaterally while in *Ctenosaura* there is a small gap here closed by the maxilla—also in *Eumeces*); in most lizards (*Iguana, Conolophus, Sceloporus, Uromastix, Gekko, Tupinambis, Cnemidophorus*) this foramen opens between the palatine, prefrontal and maxilla. In *Cnemidophorus, Anolis* and *Phrynosoma* it is largely enclosed by the maxilla while in *Tupinambis* it is a large gap between prefrontal, palatine and maxilla.

Medial to the palatine fontanelle the palatine tapers to its suture with the pterygoid. The palatine has a ventral process and a dorsal process between which the margin of the pterygoid fits. This joint is extremely variable. In *Tupinambis* there is a wide ventrolateral and a long thin dorsomedial process between which the wedge-shaped process of the pterygoid fits. In *Anolis* the palatine is broad and thin with slight transverse underlap ventrolaterally and a strong triangular process dorsomedially lapping a similar process of the pterygoid. In *Mabuya* there is a slight dorsolateral spine lying next to a spine from the pterygoid but no particular ventromedial lapping.

The palatine bears teeth in *Lanthanotus,* (some specimens of) *Heloderma,* and *Ophisaurus* (and the fossil varanid *Saniwa*).

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Vomer (Figs. 3, 4, 9, 11)

The vomers (frequently identified as the prevomers—Camp, '23: 356) are usually paired, flat plates forming the palate between the internal nares. Although usually simple plates they become laterally compressed, somewhat rolled and fairly complex in form in *Varanus*. The vomers are fused for much of their length in some iguanids (*Crotophytus, Sceloporus*), chameleons, some scincids (*Lygosoma*—unpaired in a specimen examined by Rao and Ramaswami, '52: 249, fused in a late embryo described by Pearson, '21: 44—, *Chalcides, Scelotes, Ablepharus;* not in *Eumeces* or *Scincus*), and *Gerrhosaurus* (*see* Siebenrock, 1892: 195; 1894: 252). Fusion may occur only at the anterior end of these bones in some agamids (Siebenrock, 1895: 1130—within the genus *Agama* or *Gonyocephalus* the vomers may be free or fused), in *Acontias* and some anguids (*Gerrhonotus*? but not in *Anguis* or *Ophisaurus*). Fusion of the vomers appears to be an individual or an age variation in some genera.

Parker (1880—85: 101) pointed out that the vomer was azygous even in the embryo of *Chamaeleo* but the figures of Haas ('47, pl. 1, Fig. 5; pl. 2, Fig. 7) show that the anlages are at least partly distinct. Camp ('23: 357) discussed the vomer of chameleons and pointed out that in some specimens of *Chamaeleo* there

were traces of a median suture, others had no suture. Specimens of *C. vulgaris* and *C. gracilis* had a median vomer with bilateral delicate triangular bones underlying the Jacobson's organs. The lateral elements appear to be cartilage bones identifiable with the cornu trabeculae of Parker (calcified Jacobson's cartilage of *Mabuya*—RAO and RAMASWAMI, '52: 249).

LAKJER ('27, Fig. 4) figured an embryo of *Iguana tuberculata* as having the posterior part of the vomer joined by several bony bridges, in the adult such bridges are lost.

Anteriorly the vomers usually contact the premaxilla either through some overlapping suture or by simply abuting. In *Brookesia* and *Rhampholeon* the vomers fuse with the premaxilla (CAMP '23: 358) and this may be the case in *Chamaeleo* (HAAS, '47: 200). In many lizards the vomers are partly or completely cut off from contact with the premaxilla by anterior palatine processes of the maxilla (*Tupinambis*). In *Uromastix* and chameleons the vomer passes above these processes; contact is not achieved in *Uromastix* the two bones being joined by connective tissue. In *Acontias* (a skink) and *Monopeltis* (an amphisbaenid) the anterior end of the vomers lies above the posterior margin of the premaxilla (KRITZINGER, '45: 179).

Anteriorly the vomers are constricted and shaped by the palatal openings of the Jacobson's organs. In lizards with narrower snouts the vomers tend to be rotated upwards medially to a nearly vertical position between the Jacobson's organs. The vomer may send a medial process dorsally along the cartilaginous medial nasal septum (*Cordylus,* VAN PLETZEN, '46, Fig. 4: *Lanthanotus,* MCDOWELL and BOGERT, '54: 38).

Laterally there is a gap between the vomer and the maxilla, the internal naris (and its anterior groove—*see* BELLAIRS and BOYD, '50). Medially, the vomers may diverge somewhat through the posterior two thirds of their length in agamids, iguanids (not in *Sceloporus*), varanids, *Heloderma,* xenosaurids, *Aprasia* (pygopodid), *Cordylus* (but not *Gerrhosaurus*), *Ophisaurus* and *Anguis.* There is a slight posterior notch between the vomers in almost all lizards (not in *Gekko gekko*).

The posterior margin may be drawn out as a thin process which extends back medial to the palatine (*Tupinambis, Anniella,* amphisbaenids) or it may be broad and truncated (some iguanids, geckos). Usually the palatine-vomer connection is an overlapping one, the palatine lying above the vomer either as a sheet or a tapered process. In some geckos and pygopodids the reverse situation appears to exist, a point of the palatine extends below the vomer.

Vomerine teeth are supposed to occur in *Ophisaurus* but these apparently do not fuse to the bone; none was observed in two specimens examined. In *Eumeces* (and less so in *Mabuya*) there is a ventral tooth-like process on the posterior end of each vomer next to the midline.

Septomaxilla (Figs. 1, 2, 4)

'The septomaxillary is primarily an investing bone of the vestibular wall . . . and as such appears on the outer surface of the skull in forms with a short vestibule e.g. . . . Rhynchocephalia . . . In the Lacertilia the vestibule has enormously increased in size and has carried the septomaxillary with it into the interior of the nasal organ where it forms a secondary bony roof for the organ of Jacobson . . . and causes the degeneration of the primary cartilaginous roof in all lizards with the exception of the Iguanidae (should read Chamaeleontidae). It may even cover the nasal cavity itself when the vestibule reaches back beyond the organ of Jacobson." (MALAN, '46: 126).

The septomaxilla is a shallow, inverted, cup-shaped, or nearly flat plate lying above the Jacobson's organ and forming the floor of the nasal passage. Anteriorly it contacts the maxilla and sometimes the premaxilla in the floor of the external narial opening; posteriorly and medially it rises and may contact the frontal at the midline. Processes of the bilateral septomaxillae may form a partial internarial septum. These processes appear to replace the cartilaginous internasal septum and thus meet at the midline. Anterolaterally there may be a prong of bone extending forward and upward toward the margin of the fleshy external naris.

The septomaxilla is lacking only in chameleons which retain the cartilaginous roof of the nasal capsule over the Jacobson's organ.

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Sclerotic Ring (*see* ROMER, '56, Figs. 205 B, C)

Like all primitive vertebrates (mammals, crocodilians and living amphibians excluded) the eye contains a sclerotic ring made up of a number of varyingly interlocked and overlapped bony plates. The number of plates, as in the case of the bird, varies. Only a few rings were available for counting with the following results: 11 in *Chamaeleo* (*dilepis*?); 14 in *Ctenosaura* sp? (CM. 18050), *Iguana iguana* (CM. 35157), *Anolis* sp?, *Callisaurus* sp?, *Gekko gekko* (CM. 36547), *Mabuya carinata* (RAO and RAMASWAMI, '52: 263), *Tupinambis nigropunctatus;* 15 in *Pygopus lepidopus* (UNDERWOOD, '57: 209), *Varanus salvator* (CM. 34106—all species of *Varanus,* MERTENS, '42: 132; DERANIYAGALA, '58: 162, reported 16 in *V. bengalensis*); and 20 in *Xenosaurus grandis* (BARROWS and SMITH, '46: 235).

The scleral ring of bony plates was absent in a specimen of *Amphisbaena alba* and is probably lacking in other burrowing species in which the eye is vestigial.

Stapes and *Columella* (Figs. 5, 9, 13)

The columella auris is made up of a basal stapes and a distal extracolumella. The stapes is a thin ossified rod which flares out into an ovoid basal plate which

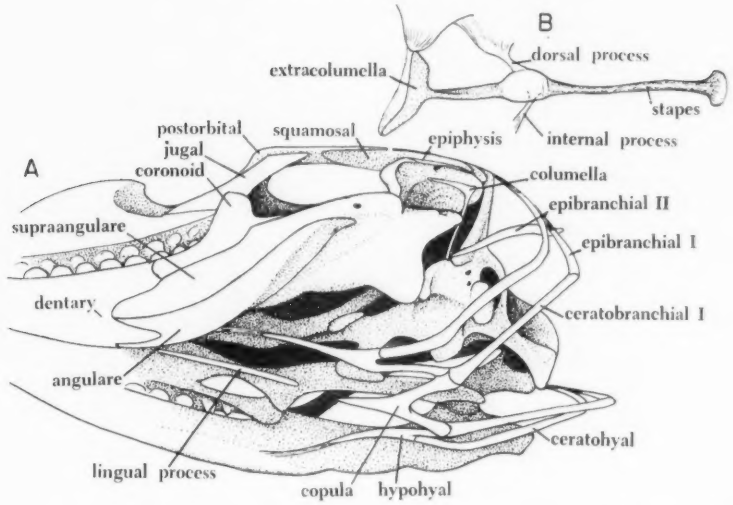

Fig. 13. Lateroventral view of the head skeleton of *Tupinambis nigropunctatus* shown in A with the second epibranchial and columella of the right side removed. B. Details of the columella as seen in ventroposterior view.

fits into the fenestra vestibuli (overlaps the margins of this fenestra in amphisbaenids). Distally it expands somewhat to join the cartilaginous (partly calcified or ossified in some) extracolumella.

The stapes is present in all lizards with the exception of *Amphisbaena ewerbecki,* in which it is unossified (ZANGERL, '44: 429—430—there is a well developed extracolumella which is partly bony), and in *Aprasia pulchella,* in which it is reduced to a minute ossicle in the membrane closing the fenestra vestibuli (UNDERWOOD, '57: 219). In *A. ewerbecki* the fenestra vestibuli is small, in other species of *Amphisbaena* it is large. In the amphisbaenids and *Anniella* the stapes is relatively large and in the former it may be quite massive with a large footplate which overlaps the region around the fenestra vestibuli. A fenestra cochleae is apparently lacking in this group. The stapes is perforated by the stapedial artery in some geckos (*Pachydactylus, Hemidactylus, Tarentola, Rhoptropus, Lygodactylus, Gonatodes, Stenodactylus, Hemitheconyx,* but not in *Gekko, Gehyra, Ptychozoon, Ptyodactylus, Ptenopus, Lepidodactylus, Saurodactylus, Uroplatus*); the stapes is notched for the artery in *Gekko vittatus* (WELLBORN, '33; VERSLUYS, 1898).

In all of the amphisbaenids the extracolumella is large and sometimes bony (or calcified—*Amphisbaena alba*); it extends forward along the outer aspect of the quadrate and attaches by a ligamentous sheet which fans out over the side of the head, particularly to the outer aspect of the mandible (*see* VERSLUYS, 1898, Tafl. 6, Figs. 66—69).

In most lizards the extracolumella is largely cartilaginous and is attached to the tympanic membrane by an elongated tympanic process (processus inferior). At the base of the tympanic process there may be a small posterior process (*Lacerta*) which is a remnant of the attachment of the hyoid cornua. Near the tip of the bony stapes is a dorsal process which extends upward between the paroccipital process and the temporal bones as the intercalare. In the adult the dorsal process is ligamentous; also a strong ligament usually attaches the base of the intercalare to the top of the tympanic process. Below the dorsal process is the internal (or quadrate) process which extends forward to attach by a ligament to the quadrate. The dorsal and internal processes are a part of the stapes, the rest is extracolumella (both parts are of hyoid origin).

"Within the Order Lacertilia the columella varies but little; . . . the internal process is lost in the Geckonidae, Uroplatidae, and Amphisbaenidae, . . . in these and a few others the ligamentous base of the dorsal process disappears, . . ." (Goodrich, '30: 452). There is somewhat more variation in the columella than this statement indicates.

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In the skink *Mabuya* (Roa and Ramaswami, '52, Fig. 10a, p. 264) or in *Scincus* (El-Toubi, '38, Fig. 3 bottom) there is no dorsal or internal process; there is a ligament from the dorsal end of the tympanic process to the region of the intercalare. *Tupinambis,* a teiid, has a small internal process (also Versluys, 1898: 69), but no posterior process at its tip. The columella is attached to the intercalare region by a ligamentous dorsal process and a ligament to the tympanic process. In *Chamaeleo* a tympanic membrane is lacking but the tympanic cavity is large and opens by a circular eustachian opening into the pharynx. The outer end of the columella is attached to the distal end of the quadrate. There is a strong ligament from the intercalare region to the distal end of the columella. There is an internal process.

Mandible

The comparative anatomy of the lacertilian mandible has been considered by Kingsley ('05). Each ramus of this structure is made up of seven bones only one of which, the articulare, is of chondral origin. The interrelationship of these bones is fairly constant, only two, the angulare and splenial, showing marked variations in size or even being absent. Some variation is induced by fusion of the supra-angulare, prearticulare and articulare.

The mandibular ramus is usually rigid; the bones overlapping each other. The more posterior bones generally fit inside of the shell of the dentary. In varanids, *Lanthanotus,* aigialosaurids and mosasaurids the ramus is capable of bending along a vertical joint between the dentary and the posterior elements. *Heloderma*

horridum appears to have a rudimentary and secondary hinge here, there is no hinge in *H. suspectum* (McDowell and Bogert, '54: 108).

The rami join anteriorly at a syndesmosis which is capable of some movement. In *Monopeltis,* Kritzinger ('45: 191) described this joint as rigid.

Dentary (Figs. 7, 8)

The dentary is the tooth bearing bone of the lower jaw, it makes up the distal half or more of each ramus. The dentaries of either side join at the symphysis (as does the partly or completely enclosed Meckel's cartilage). The inner aspect of each dentary may be open, exposing Meckel's cartilage (sulcus cartilaginis Meckelii). An open sulcus occurs in *Uromastix* (agamids and chameleons), *Varanus, Heloderma, Amphisbaena alba* and *Cordylis.* The sulcus may be partly covered by the splenial as in *Tupinambis, Lacerta, Ophisaurus, Gerrhosaurus,* and *Xenosaurus.* Various stages of closure, starting anteriorly by fusion of the dorsal and ventral margins, are observed in iguanids and scincids. The sulcus is closed in *Anolis,* geckos, pygopodids, *Xantusia, Mabuya*—i.e., in those forms in which the splenial is greatly reduced or absent, with the exception of agamids and chameleons in which the splenial is reduced or absent but the sulcus is open. Within a genus (*Lygosoma*) the groove may be largely open or closed.

The more posterior bones, generally with the exception of the coronoid, fit inside of the dentary shell. In those forms in which the lower jaw is hinged in its middle the prearticulare and the splenial overlap the dentary slightly. The dentary extends furthest posteriorly in *Anolis;* there is a broad wing on the external surface nearly to the region of the articulation.

Coronoid (Figs. 7, 8)

The coronoid is well developed and has a large dorsally projecting process. This bone straddles the dorsal margin of the ramus with a single outer flange and two internal processes. The outer process shows marked variations in form: it is lacking in *Chamaeleo,* rudimentary in *Uromastix* and very small in *Phrynosoma;* it is an elongate flange in large iguanids, *Anolis,* geckos, pygopodids, xenosaurids, *Lanthanotus,* varanids, *Heloderma, Tupinambis,* anguids, *Anniella;* a v-shaped point in *Phrynosoma, Gerrhosaurus* and *Amphisbaena;* a long, thin process directed slightly forwards in scincids, *Xantusia* and *Cordylus.*

The anterior margin of the external process may lie on a line continuous with the posterior margin of the dentary (*Lanthanotus, Heloderma,* mosasaurids, aigialosaurids, varanids, *Xantusia* and cordylids) or the posterior margin of this process may appear to be continuous with that of the dentary (*Phrynosoma, Ctenosaura,* geckos, pygopodids, xenosaurids, and *Anniella.* The scincids have a very slim process and belong with either group. The posterior margin of the

dentary may lie well behind this process in *Uromastix, Lacerta* and (the extreme case) *Anolis*. Generally this outer process overlaps the dentary slightly, or markedly.

In *Shinisaurus* there is a posterior alveolar process of the dentary which slightly overlaps the coronoid. This process is only slightly developed in most lizards and fits into the $\wedge$ between the outer and inner anterior processes of the coronoid. In *Amphisbaena alba* the dentary overlaps slightly the entire anterior margin of the outer process; the posterior margin is overlapped by the supraangulare (except at the ventral tip of the outer process where the reverse situation exists). ZANGERL's ('44) figures for *Rhineura,* and *Geocalamus* indicate that this bone may be partly fused with the others of the proximal end.

Viewed medially the coronoid is $\wedge$-shaped (except in mosasaurs), the supraangular generally shows between the anterior and posterior processes. The space between these internal processes is a fossa for the coronoid process of the palate (ventral palatine process). This fossa is only slightly developed in amphisbaenids which lack the palatine process; the notch between the anterior and posterior internal processes is very small in *Chamaeleo* or *Amphisbaena.*

The coronoid is most reduced in pygopodids, large in *Heloderma* or *Lanthanotus;* its dorsal process is tall and slim in *Anolis,* rounded and low in *Lanthanotus.*

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Supraangulare (Figs. 7, 8)

The supraangulare is frequently referred to as the surangulare. It forms the outer and dorsal portion of the proximal end of the mandible, distal to the articulatory surface. Anteriorly it laps (usually inside) the coronoid and dentary, ventrally it contacts the angulare and the prearticulare. In some lizards it is indistinguishably fused with the prearticulare and articulare (*Brookesia, Ophisaurus, Anniella,* and some amphisbaenids—*Geocalamus, Monopeltis* and *Rhineura* but not *Amphisbaena alba*).

Articulare (Figs. 7, 8)

The articulare is an ossification in Meckel's cartilage which forms the articulatory region of the mandible. This endochondral bone fuses with the surrounding dermal elements, the supraangulare and prearticulare. Fusion with the latter is complete with the result that the compound bone is frequently identified as a single element (PARKER, 1879; COPE, 1892; OELRICH, '56). Fusion with the supraangulare may be complete (gecko, *Ophisaurus*) or a suture line may remain (*Uromastix, Chamaeleo, Tupinambis, Varanus, Amphisbaena*).

The articular forms a large retroarticular process except in the chameleons, *Peltosaurus, Ophioseps* (MCDOWELL and BOGERT, '54, Figs. 22, 36) and some amphisbaenids (*Amphisbaena* but not *Rhineura*).

Prearticulare (Figs. 7, 8)

The dermal prearticulare (or goniale) fuses with the chondral articulare very early in development. Gaupp's model of *Lacerta,* figured by Wiederscheim (and Goodrich, '30, Fig. 472), shows this bone; it has also been described by Kingsley ('05), de Beer ('37: 234), and Ramaswami ('46: 284). The prearticulare extends back on the inner and ventral aspects of the mandible to sheath the retroarticular process; it extends forward inside the posterior inner process of the coronoid. The prearticulare may extend well up on the lateral surface of the mandible where it is overlapped by the angulare (*Tupinambis*) or it may be restricted to the ventral and inner aspects (*Anolis* or *Amphisbaena*).

The prearticulare may form a medial process (processus subarticularis of Siebenrock, 1895; angular process of Oelrich, '56) below the articulatory area. This may be large and wing-like as in *Anolis* and many iguanids, *Agama,* or *Tupinambis;* such a process is poorly developed or lacking in other lizards. It is a low rounded ridge in *Uromastix,* the medial angle of the posterior truncated margin in *Gekko* or *Heloderma;* this angle may be twisted upward (*Phrynosoma*) or drawn anteriorly (*Ophisaurus* or *Varanus*).

There is a large fossa on the inner aspect of the proximal end of the mandible, the ventral margin of which is formed by the prearticulare. The anterior margin of this mandibular fossa is formed by the coronoid, the posterior margin by the fused articulare and dermal elements. The mandibular blood vessels and nerves pass through this fossa and enter the ramus by way of a mandibular foramen in its anterior end. The mandibular fossa may consist of little more than the mandibular foramen and the foramen for the cutaneous recurrens nerve (*Ophisaurus,* somewhat larger in *Heloderma, Varanus, Amphisbaena*), or this fossa may be very large (*Iguana, Anolis, Uromastix, Gekko*).

Angulare (Figs. 7, 8)

The angulare is a ventral bone of the mandibular ramus, its posterior end, which extends back nearly to the articulatory surface, is wrapped up onto the lateral aspect where it overlaps the supraangulare and prearticulare. The anterior end lies along the inner ventral margin of the mandible where it is overlapped by the ventral posterior process of the dentary; a thin splint of the angulare extends forward on the ventral aspect of the ramus between the dentary and splenial (*Ctenosaura, Tupinambis, Eumeces, Lacerta, Shinisaurus, Varanus, Gerrhonotus, Ophisaurus*). When the splenial is reduced or lacking this bone extends well up on the medial surface (agamids, amphisbaenids). Its postsplenial or medial aspect portion is usually pierced by the angular foramen. The angulare of *Uromastix* (less so in other agamids) is peculiar in that the anterior end tapers to a long thin process, which extends nearly to the symphysis, between dentary

and splenial (there is no overlap on the outer aspect of this bone by the dentary). The angulare of *Lanthanotus* or a mosasaur has a truncated anterior end along the line of the mandibular hinge, this is not the case in *Varanus*.

The angulare is presumed to be absent (or fused to the prearticulare and supraangulare) in most geckos (present in *Hemitheconyx* and *Eublepharis*—WELLBORN, '33: 158) and most pygopodids while the splenial is presumed to be present (BOULENGER, 1885, I: 4; MAHENDRI, '49: 38). The situation in the gecko resembles that in many agamids or amphisbaenids in which the splenial is presumed missing. The gecko differs in that the splenial has expanded ventrally and back into the region which is ordinarily occupied by the angulare.

Amongst lizards the trend appears to be reduction of the splenial first and loss of the angular later. Only in the geckos (and pygopodids?) does it appear that the angulare is lost (whether due to fusion or not) before the splenial. In the iguanid *Phrynosoma* both angulare and splenial are equally and greatly reduced in size.

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The splenial as well as the angulare is lacking in some geckos (*Coleonyx*), pygopodids (*Ophioseps, Aprasia*), *Xantusia*, the iguanid *Anolis*, and the amphisbaenid *Geocalamus*.

Splenial (Fig. 8)

The lacertilian splenial appears to be similar to that of the bird, that is, it is a posterior splenial. The anterior splenial is possibly fused with the dentary or lacking.

The splenial generally lies completely on the inner aspect of the mandible, below the anterior leg of the coronoid. Its anterior end may extend forward nearly to the symphysis (*Tupinambis*) or this bone may be reduced in size or lacking. It is variously developed in the agamids where it ranges from small (*Uromastix aegyptia*) or minute (*Agama tuberculata*) to lacking (*Draco, Sitana, Lyriocephalus*, some species of *Agama, Phrynocephalus, Leiolepis, Uromastix hardwicki*, and *Moloch*—SIEBENROCK, 1895: 1145). Within a genus (*Uromastix* or *Agama*) this bone may be present or absent.

The splenial is best developed in the teiids, lacertids, xenosaurids, and anguids. That of the geckos is peculiar in that it occupies in part the position of the angulare. In *Gekko gekko* this bone extends forward from between the prearticulare and dentary up onto the medial aspect of the mandible to end wedged into the meckelian gap of the dentary (also in *Aristelliger, Hemidactylus, Uroplatus*).

The splenial is missing in *Anolis*, many agamids (*see* above), chameleons, *Feylinia* and amphisbaenids. According to ZANGERL ('44: 425) a splenial has been described for an amphisbaenid. In a specimen of *Amphisbaena alba*, a crevasse divided the posterior inner plate of the dentary above the meckelian groove into two parts. It may have been such a partial separation which was interpreted as

indicating the fusion of two bones, the splenial and dentary. In this same specimen, the angulare shows a crevasse which might be interpreted as evidence of a suture between angulare and splenial.

Hyoid apparatus

COPE (1892), CAMP ('23), GNANAMUTHU ('37), BEEBE (44, '45) and SONDHI ('58) have treated this apparatus (and its musculature) in particular detail. Three pharyngeal arches are represented as well as an entoglossal structure: these are the hyoid arch, part of which forms the columella, and the first and second branchial arches. The various descriptions of the hyoid appear to be based largely upon skeletal preparations and show variation in terms of presence or absence of parts. These discrepancies are due more to inaccuracies in observation than to variation within specimens, therefore, the following comparative remarks are only tentative and await revision based on dissections (aided by clearing and staining). The extent of ossification in this structure has also been confused. It appears that only the first ceratobranchial is ever ossified, the other parts may be entirely cartilaginous or heavily calcified.

Hyoid, 1st and *2nd Branchial Arches* (Figs 13, 14)

RAO and RAMASWAMI ('52: 264, using an alizarin stained preparation) described the copula and much of the lingual process in *Mabuya* as bone (only calcified); the tip and an outer cover are cartilage. The hyoid arch has a slim hypohyal, a ceratohyal and an epihyal calcification embedded in its cartilage. There is a large basal bony first ceratobranchial and a small distal splint of calcified epihyal embedded in the cartilaginous tip. The second ceratobranchial is cartilaginous and is unconnected with its distal portion which contains a calcified second epibranchial splint.

In *Chamaeleo* (also PARKER, 1880—85) the copula is little more than the basal part of the calcified lingual process; both are enclosed in cartilage. There is a calcified hypohyal which extends well forward beyond the attachment of the slim ceratohyal. There is a long bony first ceratobranchial the free tip of which serves for muscle attachment; there is no epibranchial. The second branchial arch is missing.

The hyoids of *Tupinambis* and *Amphisbaena* are only somewhat less complex than that of *Mabuya*. *Tupinambis* has the basihyal detached completely from the lingual process (both calcified), the latter is embedded in the tongue (typical ? for teiids—*see* BEEBE, '45). In Varanus the processus lingualis ends below the thyroid cartilage and does not enter the tongue proper as suggested by SONDHI ('58). The anterior process of the hypohyal of *Tupinambis* is attached by a liga-

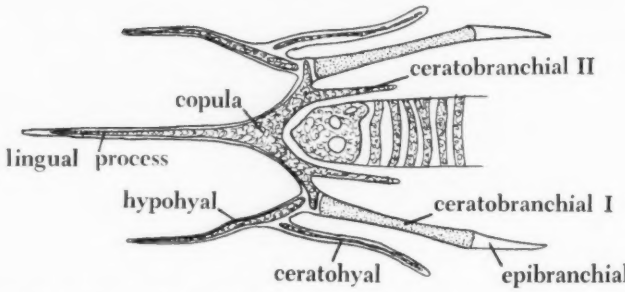

Fig. 14. Ventral view of the hyoid apparatus of *Amphisbaena alba* along with the calcifications of the trachea. Bone indicated by stippling, calcifications by marbling.

ment to the inner aspect of the mandible just above its ventral margin. In *Amphisbaena* this process is enclosed in muscle.

The hyoid and first branchial arch of *Tupinambis* are bound by a strand of connective tissue to the region of the intercalare and the second free epibranchial is attached (by ligament) to the opisthotic or exoccipital just behind the fenestra vestibuli and on the anterior aspect of the tympanic crest (as described by VERSLUYS, 1898, pl. 5, Fig. 57). *Amphisbaena* has a partly calcified epihyal; the first epibranchial is short and directed posterolaterally (like that of *Varanus*); it does not attach to the skull.

The second branchial arch is most variable. It is complete (cartilaginous) in some geckos (*Coleonyx, Sphaerodactylus, Eublepharis*—NOBLE, '21; COPE, 1892, pl. 3, Fig. 7); it may lack a segment between the epibranchial and basal portion (some geckos—*Gonatodes, Hemitheconyx*—, some pygopodids—*Delma, Pygopus*—, Xantusia, lacertids, some teiids?—*Bachia, Neusticurus, Tretioscincus*—, scincids, *Cordylus, Chamaesaura*); only the epibranchial portion may be present (some geckos—*Uroplatus, Pachydactylus*—, some teiids—*Tupinambis, Cnemidophorus, Ameiva*—, *Gerrhosaurus, Gerrhonotus, Heloderma*); only the basal portion (ceratobranchial) may be present (most iguanids and agamids—FÜRBRINGER described an epibranchial in *Iguana* and *Uromastix* according to CAMP, '23: 339—, some geckos—*Aristelliger, Platydactylus, Ptenopus*—, some amphisbaenids—*Amphisbaena, Rhineura*); or it may be lacking entirely (*Amphibolurus*, chameleons, some geckos—*Cnemaspis, Gehyra,* and *Hemidactylus* according to CAMP, '23, but not WELLBORN, '33—, *Xantusia,* a few scincids—*Acontias, Typlosaurus, Scincus*—, dibamids, *Xenosaurus, Varanus, Ophisaurus, Anguis, Anniella,* and perhaps the amphisbaenid *Bipes*—COPE, 1892, pl. 6, Fig. 43). In most iguanids (but not *Phrynosoma*) and agamids (*Agama* or *Calotes* but not *Clamydosaurus*) the second ceratobranchials extend directly posteriorly, side by side; the tips may fuse. These ceratobranchials extend back to the posterior margin of the sternum in *Anolis* and are reduced to slight processes from the posterolateral margins of the copula in *Phrynosoma.*

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The hyoid arch is lacking in dibamids and perhaps the amphisbaenid *Bipes* (Cope, 1892); a rudimentary one can be expected in some individuals. In *Rhineura* and *Anguis* only the cartilaginous hypohyal is figured by Cope; a hypohyal and ceratohyal (both calcified) are present in *Amphisbaena* (also Camp, '23, Fig. F, and Beebe, '45, text-Fig. 15) along with an anterior calcified process of the hypohyal. The hypohyal extends well beyond the point of attachment of the ceratohyal in *Chamaeleo, Xantusia,* teiids—*Ameiva, Tupinambis, Cnemidophorus, Neusticurus, Tretioscincus*—, *Anniella* and *Amphisbaena;* a short process is present in many geckos and pygopodids, agamids, *Scincus,* and *Ophisaurus.* Such a process is absent in iguanids, *Lacerta, Mabuya* and *Varanus.* In *Varanus* the ceratohyal is detached from and lies above the hypohyal and, in the region of overlap, both parts have broad, irregular margined, leaf-like ends.

Attachment of the hyoid apparatus to the skull is of interest since primitively the hyoid attached to the extracolumella (as in *Sphenodon*). Camp ('23: 340—341) has described this; his sentences are rearranged here. "Attachments of the hyoid (first arch) to the skull are known in a number of ascalabotids, in *Gerrhosaurus, Lacerta* and *Gerrhonotus,* and possibly in *Amphisbaena* . . . The primary relation of the tip of the hyoid to the ossicula auditus becomes modified in the adult form in primitive lizards by dorsal migration or looping of the remaining portion of the hyoid extremity which finally forms a close union with the paroccipital process of the opisthotic bone. This post-primary cervical union . . . is known in many geckos including *Coleonyx* (2 species), *Gekko verticillatus, Platydactylus mauritanicus, Pachydactylus bibroni, Uroplates fibriatus, Uromastix spinipes* and *U. acantherinus* . . .

"The second epibranchial is connected with the skull in the most primitive forms,—*Coleonyx* and many other Gekkonidae, *Uroplates, Xantusia, Lepidophyma, Trachysaurus, Gerrhosaurus,* and *Gerrhonotus*—being attached to the paroccipital in *Coleonyx* and *Thecadactylus,* in *Gonatodes, Lepidoblepharis, Lathrogecko, Sphaerodactylus,* and *Lepidophyma,* and to a tubercle on the exoccipital in *Trachysaurus, Tiliqua, Egernia,* and *Gerrhosaurus.*"

The first epibranchial is usually not attached to the skull (*Amphisbaena alba* or *Varanus*) but in *Tupinambis* its anterior tip is lightly bound to the epihyal which is attached to the skull. The attachment of the second epibranchial of *Tupinambis* is much like the exoccipital attachment described by Camp.

Chondrocranium

Study of the chondrocranium is made extremely difficult because of the wide range of variation shown in the several classes, the number of terms which are seemingly promiscuously applied, and by the sometimes marked changes in it which occur during development. de Beer ('37) has discussed the chondrocra-

nium of vertebrates in great detail. He has reviewed this structure as it occurs in lizards but he has not made understanding it easy. In contrast, ROMER ('56) has oversimplified, if anything, the story of the development and the changes in form of the endocranium. Attempts to make some sort of comparative meaning out of the many details usually described are numerous; that of GOODRICH ('30) is the best known. Some attempts to better understand this structure have lead to diagrammatic figures such as that for reptiles prepared by Versluys (in IHLE, VAN KAMPEN, NIERSTRASZ and VERSLUYS, '27, Fig. 374; revised by VERSLUYS, '36, Fig. 562). Gaupp's account of *Lacerta* and Versluy's diagram serve as starting points.

The base of the chondrocranium is made up of a trabecula communis in the orbital region. Anteriorly the nasal capsules attach to the trabecula communis while posteriorly its bilateral components pass lateral to the hypophyseal fenestra and attach to the anterior ends of the parachordal cartilages at the point of contact of the pila antotica (or pila prootica). The parachordals give rise laterally to the basipterygoid processes (in the region of the polar cartilages of some other forms) and the crista sellaris medially. The latter forms the dorsum sellae and cuts off the hypophyseal fenestra from the medial basicranial fenestra which separates the parachordals anteriorly.

Posteriorly the parachordals fuse to form a single plate on the dorsal surface of which lies the partly embedded notochord. Posteriorly the parachordal plate attaches on either side to the occipital arches (usually three in number) while more anteriorly it attaches broadly to the auditory capsules. Posteriorly the auditory capsules become joined by a synotic tectum which develops a medial, anteriorly extending ascending process. Later the occipital arch fuses dorsally to the posterior aspect of the auditory capsule to close off the metotic fissure.

In the orbit region the orbital cartilage (posterior orbital cartilage) appears (as the taenia medialis) and soon establishes contact with the pila antotica and, through the pila accessoria, with the taenia marginalis which may eventually extend from the planum supraseptal (anterior orbital cartilage) to the dorsal aspect of the auditory capsule. A pila metoptica extends down behind the optic nerve. Above the trabecula communis and anterior to the optic nerve the interorbital septum forms. It has an upward extending process, the pila preoptica, and joins the pila metoptica through a midline taenia hypochiasmatica. This midline structure may extend beyond the connection with the pila metoptica as the subiculum infundibuli. The interorbital septum may not have a distinct preoptic pila but it usually contacts the (anterior) orbital cartilage in front of and behind a fenestra septalis.

This is the developmental story for *Lacerta;* it is not really typical of lizards although sometimes this is assumed. The orbital wall of the "typical lizard" can more aptly be described as being formed from an anterior component (planum supraseptale), which later attaches to the interorbital septum and the nasal cap-

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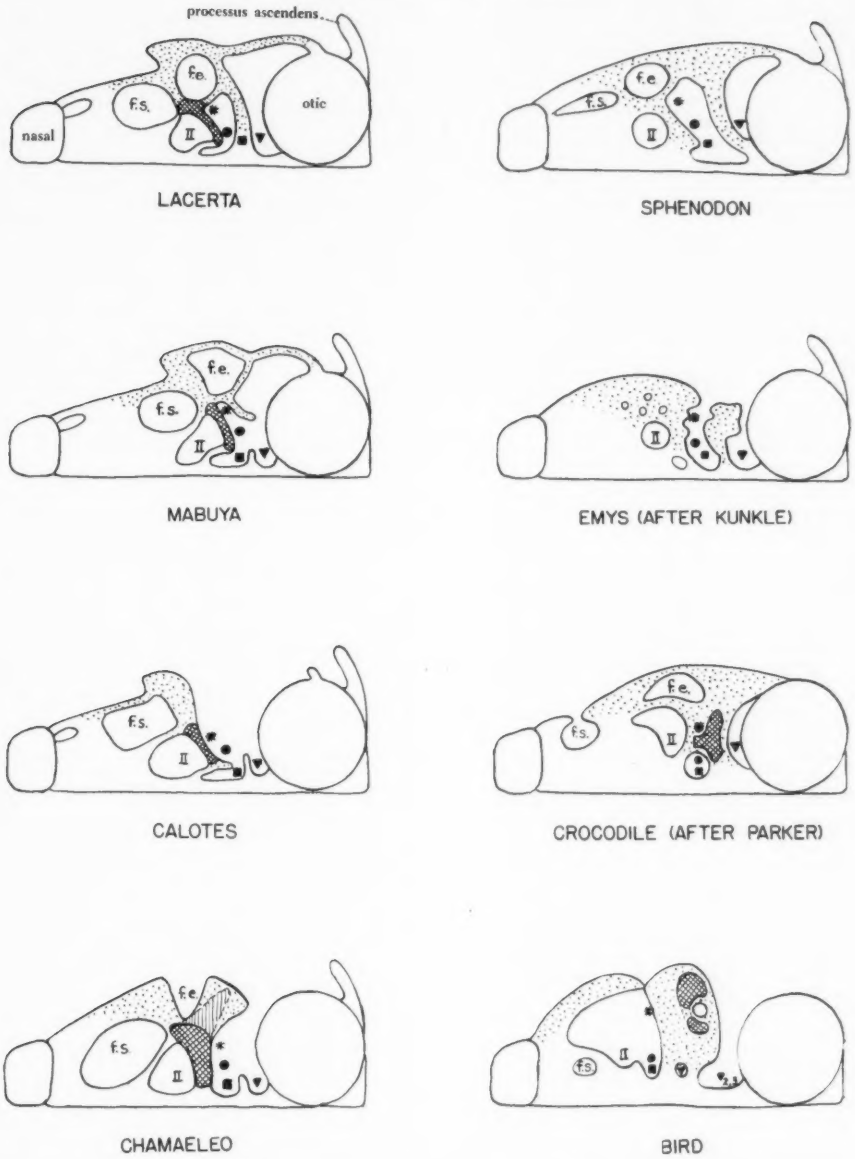

Fig. 15. Diagrammatic representations of lateral views of the chondrocrania of representative lizards, other reptiles and birds. Symbols: II — optic nerve, * — trochlear nerve, ● — oculomotor nerve, ■ — abduscens nerve, ▲ — trigeminal nerve or parts; f.e. — ethmoid fenestra, f.s. — septal fenestra. Cross hatching indicates orbitosphenoid bone, stippling the orbital cartilage. Nasal capsule to the left, otic capsule to the right.

sule, and a posterior component which usually is anchored through the pila metoptica, but which may become attached through the pila antotica to the cranial stem (later it may attach to the anterior aspect of the auditory capsule above the trigeminal foramen). The two orbital components meet and form an orbital laticework or a fairly solid plate (some living amphibians). The orbital components are joined by varying amounts of roofing in lower vertebrates.

The relationships of the nerves to the various fenestrae of the orbit walls of the lizard are shown in figure 15, as is some of the variation of the development of the orbital latice. The position of the orbitosphenoid is also indicated. Some of the variations shown are not really understandable. Attention is called to SÄVE-SÖDERBERGH's ('47) figure of *Varanus*. If the down projecting finger from the taenia marginalis is the dorsal end of the pila antotica then the fenestra metoptica is very large, a supposition supported by the position of the trochlear nerve. BELLAIRS ('40—50) figured the subadult endocranium of *Varanus monitor* in which no such descending finger of cartilage is present. Presence in one and lack in another species of *Varanus* suggests this feature is a secondary development. *Varanus* is thus like *Tupinambis*.

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Reduction of the orbital latice is greatest in the amphisbaenids (KRITZINGER, '45: 176), the orbital cartilage being replaced by the orbitosphenoid bone. Generally reduction appears to affect the pila antotica first (absent in *Agama*, geckos —*Platydactylus, Lygodactylus, Eublepharis, Gekko*—, *Tupinambis*, and *Varanus;* a basal stub occurs in the young of *Calotes*, the dorsal taenia medialis portion in *Eumeces, Mabuya*, and *Lygosoma*). SÄVE-SÖDERBERGH ('47: 500) has commented that "phylogenetically it is clear that the system of cartilaginous (and bony) bars is the result of strong reduction, first of the ossification, then also of the chondrification of this region. It is ossified to a much greater extent both in primitive fossil Tetrapods . . . and in their closest relatives among Fishes . . ."

Because of the evidence of reduction it seems best to put little emphasis on the multitudinous minor variations of the orbital cartilage. The pila metoptica and the pila antotica appear to have some comparative meaning; the pila antotica spuria of the bird being a secondarily developed substitute for the latter. A desire for a less complex (as applied by Gaupp to the chondrocranium of *Lacerta*) and more meaningful terminology appears to be shared with Säve-Söderbergh and Romer.

Variation in the cranial base ranges from the typical tropibasic condition with a trabecula communis to a platybasic type (like the snake) with separated trabeculae in the amphisbaenids (KRITZINGER, '45: 178).

Discussion

Although there is evidence of considerable variation in the form of the major units of the head skeleton, much of it explainable in terms of adaptive modification, there is an underlying consistency of pattern which seems to unit all of the many types of lizards. I am in perfect agreement with BROOM ('35: 14) that "... the Lacertilia is one of the most natural groups in the animal kingdom and that all the lizards are closely related..."

Characterization of the lacertilian head skeleton is difficult. The streptostylic quadrate, lack of quadratojugal, and the parieto-supraoccipital hinge which involves a processus ascendens appear to characterize this group. Reduced premaxillaries, which usually fuse medially, along with the general trend for medial fusion of frontals and parietals and a secondary hinge between frontal and parietal mark this group also. The pila metoptica orbitosphenoid is quite distinctive.

The lizards differ from the snakes (including Typhlopidae and Leptotyphlopidae) in that the latter have a mesokinetic skull and the parietals have enclosed the brain laterally replacing the orbitosphenoids, further the supraoccipital has been excluded from the foramen magnum in the snakes by the exoccipitals and the bones of the upper and lower jaws are moveable as a response to the needs of swallowing large prey.

Comparing the lizard type of head skeleton with that of other living reptiles—turtle, alligator and *Sphenodon*—one finds that all are specialized to some extent and none is strikingly more primitive in the totality of its structure than the others. For example, none of these types retains the interparietal bones of the primitive reptile and all show reduction in the temporal series (supratemporal, tabular), lacking one or both of these bones. Further there is a trend for reduction of the sphenethmoid ossifications. For example, the turtle skull while it lacks temporal fenestrae is yet highly modified in terms of cranial roof emargination, loss of bones (lacrimal), and modification of the cranial wall. The lacertilian in its cranial structure is close to the snake and also quite similar to the rhynchocephalian but quite unlike the turtle or crocodilian in most details.

Although there is no intent to indulge in systematic speculations in this paper, certain summarizing comments do not seem out of place. Speculating on the form of the primitive lizard one can assume that there was only moderate development of the orbital and temporal arches. Reduction of these is then a modification or a move away from the ancestral condition as is also an increased size of these arches (larger fossae). The ancestral form was a terrestrial carnivore, probably of small size. Terrestrial, arboreal, or marine plant eaters of large size are specialized as are burrowers and snake-like forms. Loss of particular bones is always indicative of modification whereas size and proportion changes in a bone can only be interpreted in terms of known trends—admitting the possibility for reversion or divergence.

In terms of skull structure the iguanids are certainly as unmodified as any lizard but not markedly more so than something like the teiid, *Tupinambis*, or the xenosaurids (*Xenosaurus* or *Shinisaurus*). If there are two basic lines of lizards, the Ascalabota and Autarchoglossa, as CAMP ('23) suggested or if there are several ascending lizard groups (Iguania and Nyctisauria, Leptoglossa and finally Diploglossa) as BROOM ('35) or ROMER ('56) suggested, these are not indicated by the head skeleton.

The larger iguanids appear to be modified in terms of the exaggerated temporal and post-temporal arches (also contact of jugal and squamosal). Some iguanids such as *Phrynosoma* show very marked divergences from the rest of the group. Similar extremes are observed in the agamids. The agamids as a group show divergence from the presumed ancestral type in further reduction of the premaxilla, tendency to changed snout bone interrelationships, reduction of the dermal superior anterior process of the prootic, reduction of the epipterygoid and loss of the splenial.

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In dentition the agamids and chameleons (some amphisbaenids) are the most modified of lizards (from the basic pleurodont type), yet in other respects these are purported to be "primitive" forms. The chameleons show many highly modified skull features which place them in a position as far from the presumed ancestral condition as that occupied by burrowing forms which have lost the temporal and sometimes the orbital arches.

I can agree with Camp, or Broom, that the chameleons are but modified agamids. In the chameleon the premaxilla has been most reduced, the snout bones are quite modified in their interrelationships, the parietal foramen has been moved between the frontals, the prefrontal and postorbital have met above the eye in *Chamaeleo* to form a supraorbital fenestra or to roof the orbit solidly. The epipterygoid and parasphenoid rostrum are lost while the orbitosphenoid is better developed than in most lizards. The pterygoid is highly modified in the spatulate coronoid process and lack of contact with quadrate. The ear is modified in terms of loss of the tympanic membrane, attachment of columella to the distal end of the quadrate and reduction or loss of the fenestra chochleae. The splenial has been lost and the hyoid apparatus is unique in lacking the first epibranchial.

The geckos and pygopodids agree in many respects and appear to represent climbing and fossorial divergences from a common group. The geckos are supposed to be primitive lizards but one could as easily describe them as highly specialized or modified with many degenerate (simplified) features. The idea that they represent an older, or if you wish earlier, development from the lizard line than any other living group is possibly true. Certainly the iguanids and agamids have more in common with other lizards than with the geckos.

The age of the lacertilia is undoubtedly great. The fact that the aigialosaurids, dolichosaurids and mosasaurids were already highly evolved in the Cretaceous and that these fossils are presumed to be related to the varanid lizards of today

(Camp, '23; '42—following Dollo, Nopcsa, and Williston) suggests that the basic radiation of the group had already occurred. The skull does not give convincing evidence of varanid affinity of these early lizards but the very diversity of their structure does support an early radiation making possible such a phylogenetic relationship (*see* discussion in McDowell and Bogert, '54: 54—57, 61—62).

On a somewhat different taxonomic plane, the head skeleton suggests that the zonurids and gerrhosaurids should not be placed together. McDowell and Bogert ('54: 98—102) have summarized many features in support of their union but fail to give any weight to such differences as exist in the hyoid structure, frontals paired or fused, presence of absence of palpebral, anterior fusion and separation of vomers posteriorly, pterygoid teeth present or absent, and the many other little details by which these groups appear to differ. Broom ('35: 19) commented on the apparent similarity of these two families and indicated the belief that they "are not at all nearly related . . ."

Zangerl ('44: 453) viewed the heterogeneous amphisbaenids (three subfamilies—Romer, '56) as quite divergent from the lizards and snakes and perhaps representative of a separate order. The head skeleton indicates they are only highly modified lizards. In spite of the radical change in the originally metakinetic skull they have retained the processus ascendens of the supraoccipital. This feature stamps the lizard as well as any; however, there are supporting details such as the teeth, foramen in the proximal end of the quadrate, the form of the vomers and septomaxilla and the details of the lower jaw and hyoid.

The development of the orbitosphenoid is the most divergent feature of the amphisbaenid yet it is not snake-like or reminiscent of any other group of reptiles. The lack of oculomotor, trochlear and abducens nerves or foramina (also vestigial condition of optic nerve and lack of chiasma) is also unique but again it is not indicative of other affinities only evidence of adaptive modification. A study of the development of the cranial skeleton in this group is needed.

The leptoglossan-diploglossan schism, which is documented by a list of some 21 features by McDowell and Bogert ('54: 102—104), is not supported by the skull features as described here. A reexamination of the many features listed by McDowell and Bogert eliminates all of those attributed to the head skeleton with perhaps the exceptions of the comments on tooth replacement and the form of the groove for the ophthalmic vein which passes above the basipterygoid process ("basipterygoid process not connected with the roof of the entocarotid fossa." McDowell and Bogert, '54: 104).

Comparing these two groups one notes that the hyoid of *Tupinambis* is much like that of *Anguis* in lacking the second ceratobranchial; or, fusion of the premaxilla is the rule (skinks being the exception) in both groups. The parietals are fused in both groups, and the meckelian groove may be open anterior to the splenial in skinks and *Tupinambis* as well as in the anguimorphs.

Seeking more sharply characterizing features one turns to other areas. On the

basis of the names of these two groups, one might assume that features of the tongue clearly distinguish them, but CAMP ('23: 374) indicated otherwise. Describing the varanid and ophidian tongue he stated that "Various stages illustrating the steps leading up to such a highly modified state may be seen in certain forms (Lacertidae, Teiidae, and Anguidae) where the tongue functions both as a masticatory and as an external tactile organ." BOULENGER (1885, II: 330) pointed out that the tongue is sheathed (i.e., like *Varanus*) in some teiids (including *Tupinambis*), not sheathed and with a bifurcate posterior margin in others. The illustrations of ROOIJ ('15, Fig. 1) and MCDOWELL and BOGERT ('54, Fig. 29) along with observations on the tongue of *Tupinambis, Heloderma* and *Amphisbaena* do not show any consistent differences between these groups. This is not an argument that they should be merged, it merely emphasizes the apparent morphological overlap in many cranial features.

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Summary

The cranial skeleton of the lizard is investigated as a part of a study of the vertebrate head skeleton starting with the mammals and working in a retrophylogenetic direction. It has been assumed that only in this way can the history (and homology) of each unit be determined. Each unit of the cranial skeleton is described in terms of its variation among lacertilians. The problem of the orbitosphenoid is discussed at some length with the conclusion that basically only two orbital ossifications occur in vertebrates, an anterior mesethmoid and a posterior (near the optic nerve) orbitosphenoid. Although there is a wide range of variation in many parts of the head skeleton, a basic lacertilian pattern is apparent. This pattern is close to that of the snake, and *Sphenodon;* quite different from that of the alligator or turtle. This pattern is quite specialized as compared with the primitive reptile, or the mammal-like reptile. However, the lizard possesses no primary bones not found in other reptiles and their mammalian derivatives; secondary bones such as epiphyses and sesamoids are not meaningfully matched in other classes. The lizards, like birds or mammals, show a radiation rather than an anatomical hierarchy of groups.

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TABLE 1

Specimens examined in the Carnegie Museum of Pittsburgh (CM followed by catalog number) as well as literature references to particular genera and families of lizards arranged after the system of Romer ('56). In brackets is indicated the number of species in a genus examined (when this seems important). An * indicates a specimen dissected.

Iguania

Iguanidae

Iguana iguana—CM 9104, 35157, 34099, one not cataloged.

Conolophus subcristata—CM 9719.

Amblyrhynchus cristatus—CM 30106.

Ctenosaura sp?—CM 35155, 35926, 18050.

Ctenosaura pectinata—Oelrich, '56.

**Dipsosaurus dorsalis*—CM 1392.

Phrynosoma coronatum—CM 33964.

Phrynosoma solare—CM 4298.

Sceloporus undulatus—CM 28655.

**Anolis* sp?—CM 33956(a), preserved specimen and skeleton in writers possession.

**Callisaurus* sp?—preserved specimen in writers possession.

Agamidae

Uromastix aegyptia—CM 9115; Beddard, '05a; Lakjer, '27; El-Toubi, '45.

Uromastix aegyptia, U. acanthinura, U. hardwicki—Saksena, '42.

Draco (1), *Sitana* (1), *Lyriocephalus* (1), *Gonyocephalus* (4), *Japalura* (1), *Calotes* (5), *Psammophilus* (1), *Agama* (8), *Phrynocephalus* (2), *Amphibolurus* (3), *Lophura* (1), *Leiolepis* (1), *Uromastix* (2), *Moloch* (1)—Siebenrock, 1895.

Calotes versicolor—Ramaswami, '46.

Amphibolurus, Physignathus, Clamydosaurus—Beddard, '05b.

Agama stellio—El Toubi, '45.

Chamaeleontidae

**Chamaeleo (dilepis?)*—CM preserved specimen.

Chamaeleo—Parker, 1880—85; Haas, '47.

Brookesia superciliaris—Siebenrock, 1893a.

Microsaura ventralis—Brock, '40.

Nyctisauria

Gekkonidae

Gekko (3), *Gehyra* (2), *Ptychozoon* (1), *Ptyodactylus* (1), *Tarentola* (2), *Rhoptropus* (1), *Hemidactylus* (1), *Ptenopus* (1), *Lygodactylus* (1), *Lepidodactylus* (1), *Gonatodes* (1), *Stenodactylus* (1), *Saurodactylus* (1), *Ebenavia* (1), *Hemitheconyx* (1), *Uroplatus* (1)—Wellborn, '33.

Gekko gekko—CM 36547.

Aristelliger, Coleonyx—McDowell and Bogert, '54, Figs. 23, 24.

Hemidactylus flaviviridis—Mahendri, '49.

Uroplatus fimbriatus—Siebenrock, 1893b.

Lygodactylus capensis, Pachydactylus maculosa—Brock, '32.

Pygopodidae

Pygopus, Delma, Lialis, Aprasia, Ophioseps—McDowell and Bogert, '54, Figs. 17—22.

Pygopus, Delma, Lialis, Aprasia—Underwood, '57.

Leptoglossa

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Xantusiidae

Xantusia riversiana—McDowell and Bogert, '54, Fig. 25.

Teiidae

Teius, Callopistes, Cnemidophorus, Ameiva—Broom, '35.

Cnemidophorus sexlineatus—CM 4840.

Tupinambis teguixin—CM 9992.

**Tupinambis nigropunctatus*—CM 35162, fresh specimen, 2 head skeletons in writers possession; Reese, '23.

**Ameiva ameiva*—CM 933.

**Ameiva thoracica*—CM 34140.

**Ameiva dorsalis*—CM 4309.

**Ameiva exul*—CM 36039.

**Ameiva atrigularis*—CM 6505.

Scincidae

Eumeces (1), *Lygosoma* (9), *Mabuya* (5), *Ablepharus* (1), *Chalcides* (5), *Scincus* (1), *Tilique* (3), *Egernia* (1)—Siebenrock, 1892.

Eumeces—Taylor, '35; Kingman, '32; Rice, '20.

Eumeces laticeps—CM 32056.

Mabuya carinata—Rao and Ramaswami, '52.

Chalcides, Ablepharus—Haas, '36, '35.

Lygosoma—Pearson, '21.

Tiliqua niger—Huxley, 1864.

Lacertidae

Lacerta (11), *Takydromus* (1), *Psammodromus* (1), *Algyroides* (1), *Acanthodactylus* (1), *Eremias* (2), *Ophisops* (1)—Siebenrock, 1892.

Lacerta, Poromera, Algyroides, Nucras, Latastia, Gastropholis, Holaspis—Degen, '11.

Lacerta agillis—Gaupp, '00; Parker, 1879.

Cordylidae

Gerrhosaurus (1), *Zonosaurus* (2)—Siebenrock, 1892.

Zonurus, Platysaurus—Broom, '35.

Cordylus, Gerrhosaurus—McDowell and Bogert, '54, Figs. 26—28.

Cordylus polyzonus—van Pletzen, '46.

Dibamidae—Romer, '56; Boulenger, 1887.

Diploglossa

Anguidae

Anguis (1), *Ophisaurus* (1), *Gerrhonotus* (1)—Siebenrock, 1892.

Anguis, Diploglossus, Gerrhonotus, Peltosaurus—McDowell and Bogert, '54, Figs. 36—41.

Anguis fragilis—CM 28642, 28643, 28644.

Ophisaurus attenuatus—CM 32301.

Ophisaurus sp?—CM 33955.

Anniellidae

Anniella pulchra—McDowell and Bogert, '54, Fig. 42; Bellairs, '50; Coe and Kunkel, '06.

Xenosauridae

Xenosaurus grandis—Barrows and Smith, '47.

Shinisaurus crocodilurus—McDowell and Bogert, '54, Fig. 4.

Varanidae—Mertens, '42.

Varanus niloticus—CM 9736, 14990.

Varanus salvator—CM 34106.

Varanus varius—McDowell and Bogert, '54, Fig. 8.

Varanus monitor—Bahl, '37.

Varanus bengalensis—Deraniyagala, '58.

Helodermatidae

Heloderma sp?—CM 33967.

Heloderma horridum—McDowell and Bogert, '54, Fig. 7.

**Heloderma suspectum*—preserved specimen in writers possession.

Lanthanotidae

Lanthanotus borneensis—McDowell and Bogert, '54, Fig. 2.

Aigialosauridae—McDowell and Bogert, '54, Fig. 9.

Mosasauridae—McDowell and Bogert, '54, Fig. 10; Camp, '42.

Annulata

Amphisbaenidae

Amphisbaena (3), *Bipes* (1), *Geocalamus* (1), *Trogonophis* (1), *Rhineura* (1), *Monopeltis* (1), *Leposternon* (1)—Zangerl, '44.

**Amphisbaena alba*—CM 4322; Lakjer, '27, Figs. 54—56.

Amphisbaena—Romer, '56, Fig. 66A, B, C: Versluys, '36, Fig. 599A, B.

Monopeltis capensis—Kritzinger, '45.

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Innervation of the Hearts of Sepia officinalis

by

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Introduction

The circulatory system in the Cephalopoda is remarkable in that three hearts are active in the propulsion of the blood: one of them, consisting of two auricles and a ventricle, drives the blood coming from the branchiae into the arterial system of the body, while the other two, so called branchial hearts, receive the blood from the veins and pump it into the branchiae. The nerves having relation with these organs have been described by several authors and their action studied in physiological experiments, but little is known about their finer anatomy. This may be chiefly due to technical difficulties in obtaining satisfactory histological preparations of this part of the nervous system in Cephalopoda, as well as in other Mollusca. Methylene blue, although it may stain the nerves in other organs is not of much use in this instance; gold and silver methods give as a rule discouraging

results for either the nerves do not stain at all, or the background becomes so dark that the nerves cannot be distinguished in whole mounts which in this kind of investigations are of great importance. In this respect one of the modifications of Cajal's method, suggested by Schultze, has the great advantage of staining the nerves selectively while leaving other tissues fairly transparent and, as I pointed out in my work on cephalopod eye (ALEXANDROWICZ 1927), it gives good results with the organs of these animals. Some trials which were then made to stain the heart showed that its nerves also can be made visible, but I had no opportunity of continuing that work. Having resumed it in the Plymouth Laboratory I chose Sepia as the first subject for these studies and again applied the same staining procedure with considerable success. As however this method of silver impregnation has its limitations it had been supplemented by other techniques of which that of Bodian proved to be very suitable for cephalopod nerves. Methylene blue has not been abandoned and in certain cases has been of good help. The details concerning the dissection and staining procedure are given at the end of this paper.

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I wish to express my sincere thanks to Dr F. S. Russell F.R.S., Director of the Plymouth Laboratory, for his kindness and hospitality, and for his help in preparing the manuscript. I am indebted to the Royal Society for a grant enabling me to continue scientific work.

General arrangement of the nervous system of the hearts in Sepia

The disposition of elements concerned with the innervation of the hearts in Sepia and their terminology used in the present account are shown in Fig. 1. The two visceral trunks (*nn.viscerales*), the anterior parts of which issuing from the visceral ganglia are not represented, are connected by a commissure (*commissura visceralis*) from which arises the nerve trunk to the ventricle (*n.cardiacus*). Each visceral nerve proceeds backwards and slightly outwards to pass into the ganglion at the base of the gill (*ganglion cardiacum*) which sends nerves to the auricle, to the branchial heart, several others to the neighbouring organs and a ganglionic trunk (*truncus ganglionaris branchialis*) into the branchia.

The description of this system in Sepia, as found in text books and in papers dealing with this subject is based on the researches of three authors.

CHÉRON (1866) was the first to give a good description and illustration of these nerves. His work was in fact so good that not much has been added to it since.

JATTA investigated this system too. His results however became known only from the drawing he left and which were published by other authors among them by BAUER in his "Einführung in die Physiologie der Cephalopoden" (1909). As the

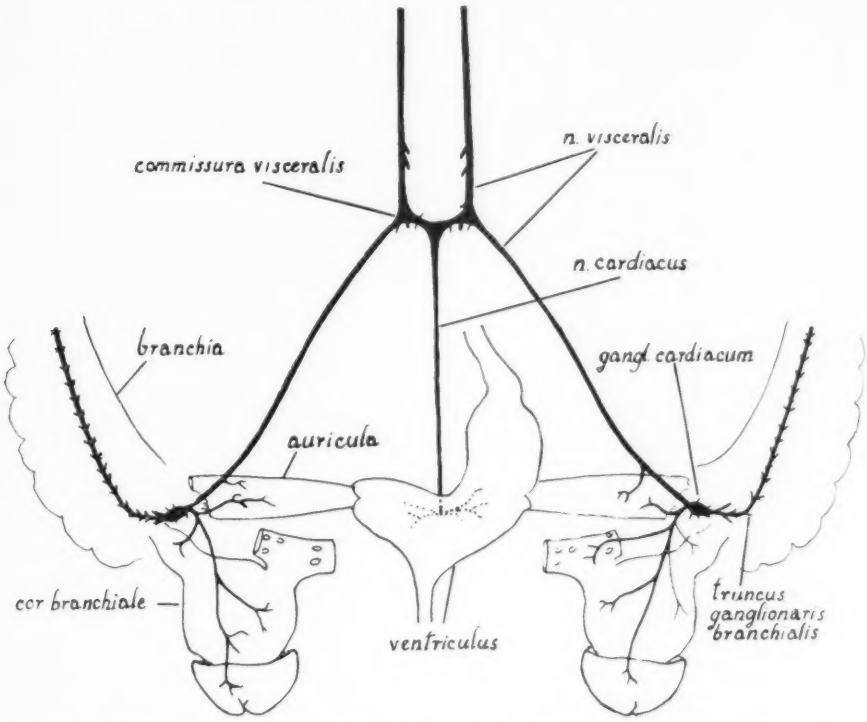

Fig. 1. *Sepia officinalis*. General arrangement of the nerves of the hearts. Dorsal view.

latter paper has been widely used as a guide by students of cephalopods, Jatta's views are reflected in several other papers.

HILLIG (1912) made a thorough study of the whole nervous system in Sepia correcting the data of previous investigators and adding many details. He gave also a comprehensive survey of the bibliography in which the conceptions of various authors concerning the heart innervation are discussed.

There is an unwelcome discrepancy in the terminology employed by different writers. Thus the term nervus branchialis is used by some of them for designating the postcommissural part of the visceral nerve. This term however is misleading, firstly because this nerve has relations not only with the branchia but also with other important organs, and secondly, because the designation n.branchialis has been applied, with better reason, to the nerve trunk within the branchia. It seems therefore advisable to retain the term "visceral nerve" for the trunk up to the cardiac ganglion.

The visceral commissure is called by Jatta "first heart ganglion". Hillig dicagrees with this designation and for reasons explained below, it is better not to use it.

Hillig distinguishes two visceral commissures, the anterior and the posterior one, the former consisting of anastomoses between the branches of the visceral nerves running to the rectum and to the inksac. The occurrence of such anastomoses can be confirmed by the present writer, but is seems preferable to reserve the term "visceral commissure" for the

trunk linking the visceral nreves and not to use it for connections between their branches. Hillig himself points out that his additional "first commissure" cannot be compared with the main one, i.e. his "posterior commissure".

The ganglion at the base of the branchia, our cardiac ganglion, was called by Chéron "ganglion of the branchial heart", by Jatta "second heart ganglion", and by Hillig "branchial ganglion (Kiemenganglion)". As it has relation not only with the branchial heart, but also with the ventricle and the auricle, the more general term cardiac ganglion is adopted. Jatta's addition "second" is unnecessary after the term "first heart ganglion" has been dropped. The term "branchial ganglion", used by some authors already before Hillig, is certainly ill-chosen as this ganglion takes only a small part in the innervation of the gill and, moreover, this term, as has been pointed out by PFEFFERKORN (1915), is inacceptable from the standpoint of comparative anatomy, since in the Octopoda he homologous ganglion is situated at a distance from the gill and the latter has its own small "branchial" ganglia.

For the trunk penetrating into the gill the term branchial ganglionic trunk is proposed. Hillig, calling it nervus branchialis, uses the same term as for the postcommissural part of the visceral nerve not taking into account the fact that the trunk in the gill has a fundamentally different structure for it contains a great number of ganglion cells, and in the Octopoda it consists of a row of small ganglia, both these features correctly observed by Chéron. The term ganglionic trunk fits well in both cases.

In Jatta's figure (v. BAUER 1909) a swelling of this trunk near the cardiac ganglion is shown and designated as branchial ganglion. Among many preparations examined it was only rarely that a slight swelling at this place could be noticed, but it was not so conspicuous as in Jatta's figure. Anomalies in shape and position of the cardiac ganglion do occur and it is possible that Jatta's drawing was made from a preparation exhibiting unusual features. Some deformations are also not uncommonly produced during dissection and fixation.

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Descriptive

As may be gathered from the general disposition of the heart nerves, in order to gain a better knowledge of this system, it was necessary to examine: 1. the cardiac nerve and the innervation of the ventricle; 2. the innervation of the auricles; 3. the innervation of the branchial hearts, and 4. the cardiac ganglion.

The following description and all the illustrations refer to Sepia officinalis. It will be remarked that the preparations shown in photographs were made from specimens of various sizes.

INNERVATION OF THE VENTRICLE

Nervus cardiacus

The cardiac nerve originates from the middle part of the visceral commissure with several bundles (Figs. 2, 3, 4). The fibres constituting these bundles reach the commissure from two opposite directions: one set of them, issuing from the visceral ganglia, travels down in each visceral trunk and on reaching the commissure converges towards the median line to pass into the cardiac nerve (Fig. 2a); the second set, arising from the cardiac ganglion, ascends in the visceral trunk

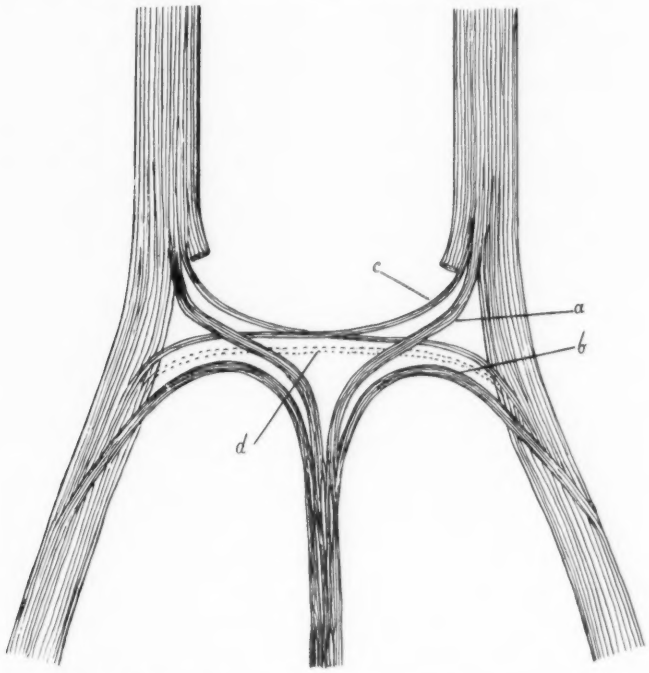

Fig. 2. Diagram showing the fibres from the central nervous system (*a*), and from the cardiac ganglion (*b*) forming the cardiac nerve. *c*, fibres decussating in the commissure; *d*, fibres presumably running from each cardiac ganglion towards that of the opposite side.

towards the commissure and curves in it like an arch to join the former in the cardiac nerve. (Figs. 2*b* and 6*b*).

The way in which the nerve bundles pass into the commissure produces a widening of its lateral parts and this may have been the reason for considering them to be the heart ganglia. It is true that nerve cells, as first pointed out by Chéron, do occur here. They are situated in small groups, mainly in the lateral parts of the commissure and in the adjoining parts of the visceral nerves, but only a few of them near the median line. The cells rarely show in methylene blue preparations, but can be easily distinguished in sections stained with azan or Bodian's method. The course of their axons however could not be determined and therefore it is not clear which organs are supplied by these neurons, but as far as can be judged from their location and their relation to various nerve bundles, it seems unlikely that they could take any significant part in the constitution of the cardiac nerve. At any rate the term "heart ganglion" for the commissure would be certainly inappropriate.

The bundles forming the cardiac nerve leave the commissure in a loose arrangement, but in their further course come more or less close together. They do not

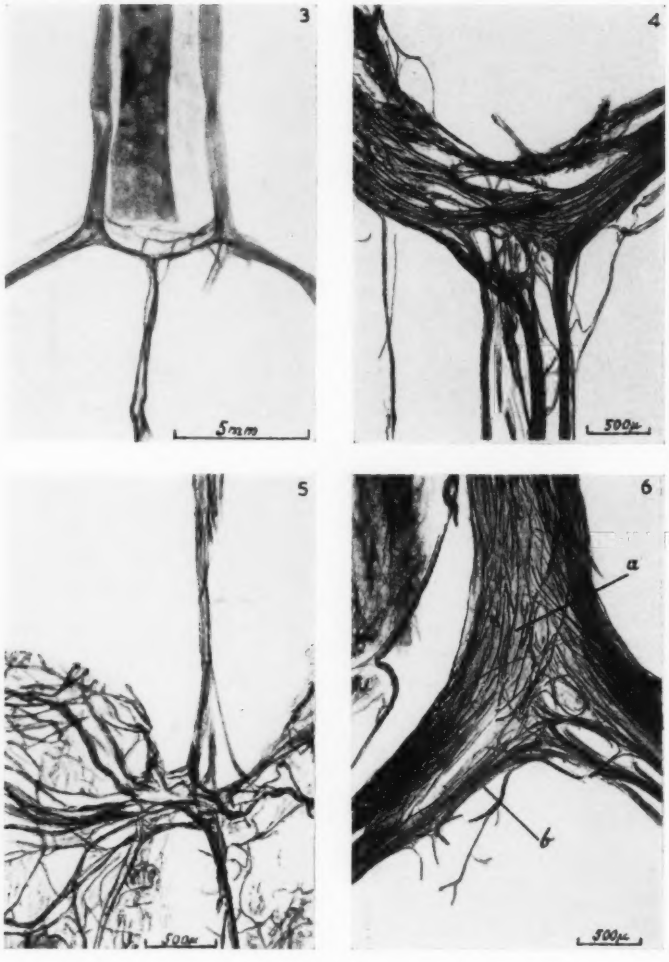

Fig. 3. Visceral commissure and cardiac nerve. The vena cephalica running between the visceral nerves has been cut through in front of the commissure.

Fig. 4. Middle part of the commissure with the origin of the cardiac nerve.

Fig. 5. Cardiac nerve passing into the main trunk of the ventricle. The part of the ventricle wall through which the nerve passes has been removed. The arrangement of the nerve bundles and the shape of the main trunk is atypical.

Fig. 6. Lateral part of the commissure. *a*, fibres from the central nervous system; *b*, fibres from the cardiac ganglion (cf. Fig. 2).

Preparation shown in these photographs were made from specimens differing greatly in size. Fig. 3, methylene blue staining; Figs. 4—6, Schultze's method.

preserve their individuality as tracts each containing elements of the same source: already in the commissure fibres may be seen passing from one bundle to another, and further on the bundles may split and exchange anastomoses. Fibres of various

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origins, i.e. from the central nervous system and from the cardiac ganglion, do not exhibit such structural differences which would allow them to be discerned in their course towards the ventricle.

The fibres are of various thickness, the stouter measuring up to 15 μ, the thinnest about 2 μ. Their total number counted on enlarged photographs of a transverse section of the cardiac nerve amounted to more than 800. This estimate is not exact as many doubts arise when counting the thin fibres, but it is certainly not higher than the real one and anyhow gives an idea of the extraordinary abundance of nerve elements supplying the ventricle. The question may well be raised whether all these fibres originate from as many nerve cells or whether their great number can be the result of frequent branching of the cell axons. The evidence available speaks in favour of the first alternative. In all the preparations in which the fibres can be followed in the visceral trunks for some appreciable distance, they can be seen running without dividing. They may do this in certain places, as in the commissure, where the branches take different directions, but splitting of a thick fibre into many branches which would run parallel to each other seems very unlikely. It is true that divisions at an acute angle in the trunk could be easily overlooked, but if a greater number of fibres resulted from such multiplication the appearance of the visceral trunk in various regions would show marked differences and near the ganglia it would consist of a small number of thick fibres only. As a matter of fact nothing of this kind could be observed.

It is difficult to estimate the relative number of fibres in each set of different origin. One has sometimes the impression that the fibres coming from the cardiac ganglion are less numerous than those arising from the central nervous system, but this may be due to the fact that out of the former set of fibres only those forming the marginal bundle (Fig. 6*b*) can be more easily followed whilst others passing through the thickness of the commissure cannot be traced with certainty. It must be realized that the structure of the commissure is a very complicated one. Hundreds of fibres brought by visceral trunks intermingle in it, many of them bifurcating here; some continue their course in the visceral trunks, others pass into several nerves arising from the commissure. In Fig. 2 all these nerves have been omitted and of the fasciculi passing through the commissure, apart from the components of the cardiac nerve, only those are shown which are likely to belong to the system concerned with innervation of the hearts. These are: *c*, fasciculi coming from the central nervous system and decussating in the commissure to run towards the cardiac ganglion of the opposite side, and *d*, fasciculi which presumably pass from the cardiac ganglion of one side to the opposite one. It must however be emphasized that no certitude as to the course of the latter could be obtained because they run through that part of the commissure where the tracing of fibres is very difficult.

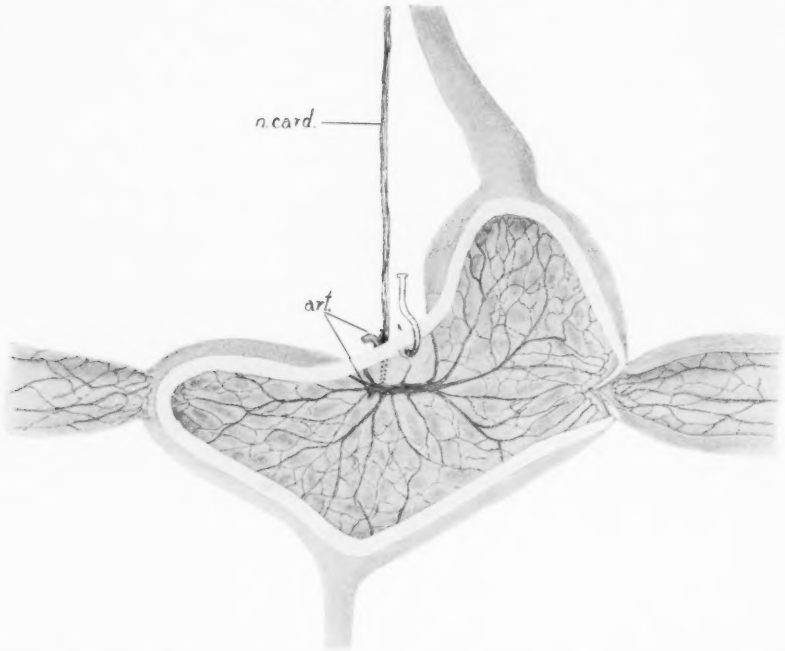

Fig. 7. The ventricle with proximal parts of the auricles. The dorsal wall of the ventricle has been cut away to show the nerves on the inner side of the ventricle. The passage of the cardiac nerve (*n.card.*) through the ventricle wall is indicated by dotted lines. *art.*, arteria genitalis posterior. The right auricle is cut longitudially and its walls pulled slightly apart.

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Nerves in the wall of the ventricle

The cardiac nerve reaches the ventricle at its anterior concave face and passing a little further on to the anterioventral wall penetrates into it close to the origin of two small arteries (*a. genitalis posterior* and *a. renalis anterior*). Near this point a few thin branches detach themselves from the nerve and ramify on the surface of the heart while all others pierce the whole thickness of the wall to appear on its inner surface as a broad trunk from which arise branches spreading in all directions (Figs. 7, 5, 8).

During the passage through the heart wall the nerve becomes more or less flattened and its fibres usually keep close together. Emerging on the inner surface most of them turn right so that their point of entrance, seen from inside, can be found at the left end of the trunk (Fig. 7). In photographs (Figs. 8, 11) it is seen to be situated on the right end because these hearts were opened from the ventral side. The aperture leading to the genital artery is also situated nearby (Fig. 7, *art.*). At the point of entrance many fibres bifurcate and there is here

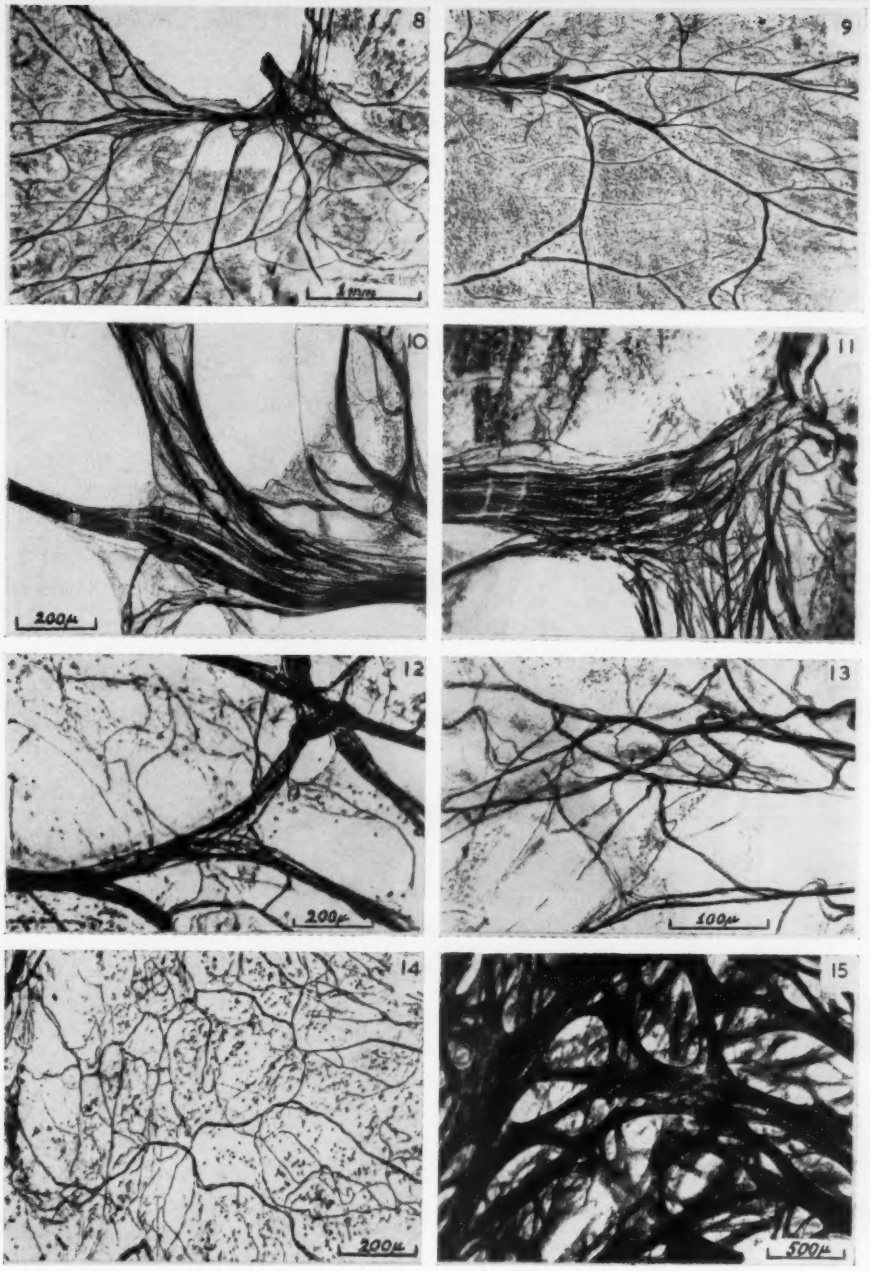

Fig. 8. Main nerve trunk of the ventricle and its branches. The entrance of the cardiac nerve is seen on the right because the ventricle has been opened from the ventral side. The muscles beneath the main trunk have been removed. The trunk is narrower than it should be because some of its fibres were severed during dissection.

Fig. 9. Part of the ventricle with nerves spreading on its inner surface.

Figs. 10, 11. Right and left ends of the main trunk in the ventricle. Orientation as in Fig. 8. The structures seen around the nerves in Fig. 10 are muscles of the inner layer adhering to the nerves. Note in Fig. 11 the branching of the fibres entering the trunk.

Figs. 12—14. Nerves in the plexiform muscle layer of the ventricle. The scale in Fig. 13 should read 500 μ.

Fig. 15. Plexiform muscle layer of the ventricle. In Figs. 12 and 13 the outlines of the same muscle layer are only faintly stained. All figures from preparations stained with Schultze's method.

an entanglement of fibres of which the photographs (Figs. 8 and 11) can give an idea.

Sometimes a less regular arrangement may be encountered in which the nerve bundles passing through the heart wall take a more or less diverging course and enter the trunk at various points (Fig. 5).

The main trunk is situated transversely i.e. parallel to the line between the apertures of the auricles. Its length in medium sized specimens is about 5 mm., but may very greatly and the trunk itself has sometimes an irregular shape.

Structure of the ventricle.—The heart wall in the greater part of its thickness consists of a compact muscle layer with some connective tissue among the muscle fibres. Near the inner surface the muscle bundles become more loosely arranged in a sort of three-dimensional plexus so that the ventricle seen from inside has a spongy appearance (Fig. 15). In the innermost layer the muscle bundles are more flattened with the exception of few long thread like bundles which being attached only at their ends span several meshes of the plexus.

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The muscle fibres are finely cross-striated as it has been stated by MARGEAU (1904). Connective tissue is represented by fine fibres and elongated cells with thin processes and small nuclei. The surface of the ventricle is covered by a layer of simple squamous epithelium.

Distribution of the nerves in the ventricle.—The main nerve trunk gives off several branches the thickest of them arising at its ends (Figs. 8—11). They spread over the whole inner surface of the heart often anastomosing with one another (Figs. 7, 9, 12, 13). The thicker trunks are all confined to the innermost layer of the muscles and adhere to them so that when these trunks are removed from the heart the flattened muscle bundles come off with them too. Into the deeper plexiform muscle layer run branches of smaller calibre covering the muscle bundles with densely distributed fibres (Figs. 12, 14). Still thinner branches penetrate into the compact muscle layer. The latter, at first sight, seems to have a very poor innervation, but this is due to the fact that the impregnation of thin fibres is as a rule much less satisfactory and there are no thicker branches here.

As mentioned before, the cardiac nerve carries fibres of various sizes and these differences can be also seen in the main trunk and stouter branches. The tracing of individual fibres through many subdivisions is so difficult that no information about the course of elements of various sorts could be obtained.

The nerve fibres branching on the muscles do not exhibit any special end-organs. One ought to be wary of interpreting preparations in which nerve fibres seem to end as showing the real limits of their course, and if a small knob happens to be there, regarding it as an endorgan. This can be nothing more than the point at which the dye ceases to become fixed by the nerve substance. In the best of preparations which I have been able to obtain by means of various methods, the finest nerve filaments, usually stained in a lighter hue, were becoming gradually less distinct until no more discernible. Observation of such features

leads to the same conclusion about terminations of nerve fibres on the muscles in Mollusca as I have previously expressed for Crustacea, namely that for the elucidation of the morphological relations of the nerves to these muscles our staining techniques and the magnification of the ordinary microscope are inadequate.

The nerves ramifying in the ventricle wall do not communicate with those of the auricles and of the arteries arising from the heart. All the somewhat thicker branches running on the inner surface of the ventricle, on approaching the apertures of these organs, turn sideways or even backwards. Only very thin branches may be seen ramifying in the muscles near the auriculo-ventricular valves and also passing on to the valves themselves; the latter thus are included in the nervous system of the ventricle. No nerve branches could be seen coursing in the other direction i.e. from the auricle or the arteries into the ventricle.

It is worth noting that there is a marked difference in staining reactions of the nerves in the ventricle on the one side and in the arteries on the other. The latter stain readily with methylene blue showing a dense plexus of fibres; staining stops abruptly at the ventricle on which the nerves, as a rule, do not show at all. In contrast, the silver methods give good results with the ventricle nerves and rather poor ones with those of the arteries.

No ganglion cells could be found in the ventricle. Admittedly, negative results in investigations of nervous system may always leave some doubts as to whether all the elements have actually been brought to view by the methods applied. In this case however the assumption that the nerve cells are missing has a high degree of probability. Considering the disposition of the nerves in the ventricle the most likely place for the location of cells would be the main nerve trunk, but neither in it nor in its vicinity could they be detected. Pictures obtained with methylene blue staining and with Schultze's silver impregnation are, of course, not conclusice since both these methods may give consistently negative results with certain nerve elements, but examination of serial sections of this part of the ventricle cut in various planes and stained with different methods has given negative results as well. It is possible that some single element might not have been spotted, but this could not be the case if more of them were present since ganglion cells in cephalopod organs are not particularly difficult to distinguish in preparations stained with azan or Bodian's method. It may therefore be assumed that in the main nerve trunk of the ventricle the ganglion cells are missing. A similar assumption however cannot have the same degree of certainly as regards the whole ventricle for some single cells in an organ of that size can be overlooked. If however only a few scattered cells were present they would be more likely to be stray elements than an important factor of innervation in which hundreds of neurons take part.

It should be emphasized that such a distribution of the ventricle nerves as that found in Sepia cannot be regarded as typical for the Cephalopoda. Some observations with *Eledone cirrhosa* have shown that in this animal the nerves reaching

the ventricle from its sides (there is no median cardiac nerve here) do not pierce the wall, but spread their thicker branches on its surface, and only their finer divisions penetrate into the muscle layer and to its inner surface. This is therefore the complete reverse of the pattern of innervation of the ventricle in Sepia.

INNERVATION OF THE AURICLES

Structure of the auricle

The wall of the auricle is thin and when stretched it is membrane-like and transparent enough to show the arrangement of its tissues in whole mounts. It consists of a layer of muscle bundles and of connective tissue spreading in the muscle layer and forming a coat on its surface. As seen in Figs. 21 and 24, the muscle bundles run in all directions crossing each other and exchanging anastomoses. They exhibit a fine cross-striation of their fibres similar to that of the fibres in the ventricle, but with the muscles of the auricle it is somewhat more difficult to obtain preparations showing this feature.

The connective tissue is characterized by the abundance of thin fibres which form a dense plexus. Its outer layer is attached at many places to the surrounding wall of the viscero-pericardial coelom; in some of the connecting strands run fine muscles, blood vessels and thin nerves passing thus from the pericardium to the auricle. It is interesting to note that the wall of the auricle, though bathed on its inside by the arterial blood coming from the gills, receives also a blood supply through the vessels coming from the outside and spreading out in its wall.

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Nerve elements in the auricle

In the innervation of the auricle the following elements take part: 1. fibres originating in the cardiac ganglion; 2. fibres issuing from the visceral ganglion; 3. axons of ganglion cells situated in the wall of the auricle. The fibres (1) and (2) associate in the same trunks and consequently the nerves to the auricle, at least the main ones, although apparently starting from the cardiac ganglion or from the visceral trunk, are actually of mixed composition.

Nerves from the cardiac ganglion.—The stoutest of these nerves arises from the cardiac ganglion together with the nerve of the branchial heart (Figs. 16, 28). The point of origin of the common root is situated near the entrance of the visceral trunk into the ganglion. The two nerves separate at varying distances from the ganglion, sometimes quite close to it.

Apart from this stout trunk there are others, of much smaller calibre, which in varying numbers take their origin from the ganglion and run to the auricle.

As shown in Fig. 17, only one set of fibres in the common root of the nerves to the auricle and to the branchial heart come out of the cardiac ganglion; other

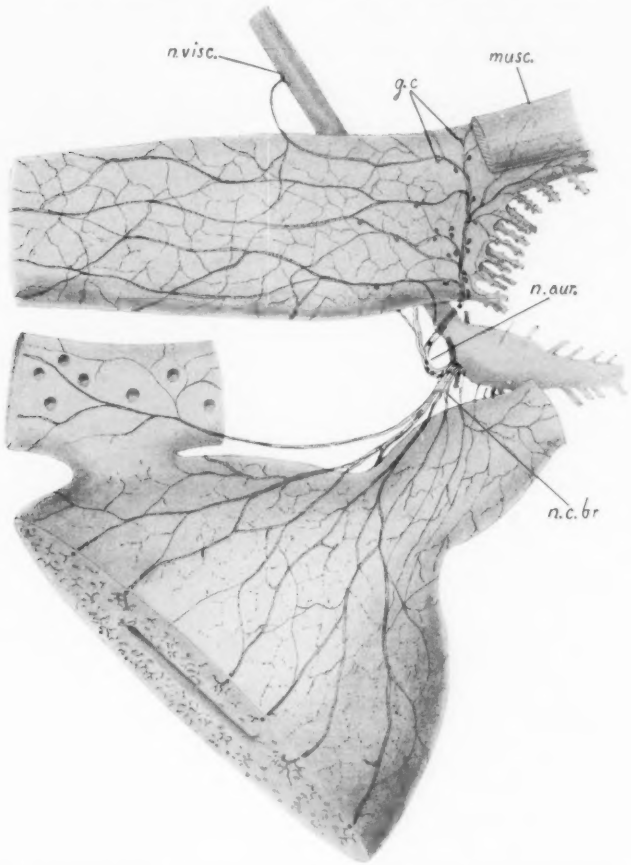

Fig. 16. Parts of the left auricle and the branchial heart pulled apart to show the cardiac ganglion. Ventral view. The auricle is slightly flattened. Note the presence of several efferent branchial vessels beside the large one (figures in text-books showing only one vessel are inaccurate). The branchial heart has been cut transversely to show the nerves in the thickness of the wall. *n.aur.*, nervus auriculae; *n.c.br.*, nervus cordis branchialis; *n.visc.*, nervus visceralis giving off the branch to the auricle; *g.c.*, ganglion cells; *musc.*, one of the portions of the retractor muscle of the branchia.

fibres, originating in the central nervous system, are carried by the visceral nerves and join the former curving into the common trunk. The mixed composition of the latter was particularly manifest in one preparation in which the cardiac ganglion was abnormally situated not at the base of the gill, but a little farther within the gill, and the common root of the two nerves made up of fibres coming from the opposite directions emerged not from the ganglion but from the visceral trunk.

It will be observed that among the fibres curving from the visceral nerve a

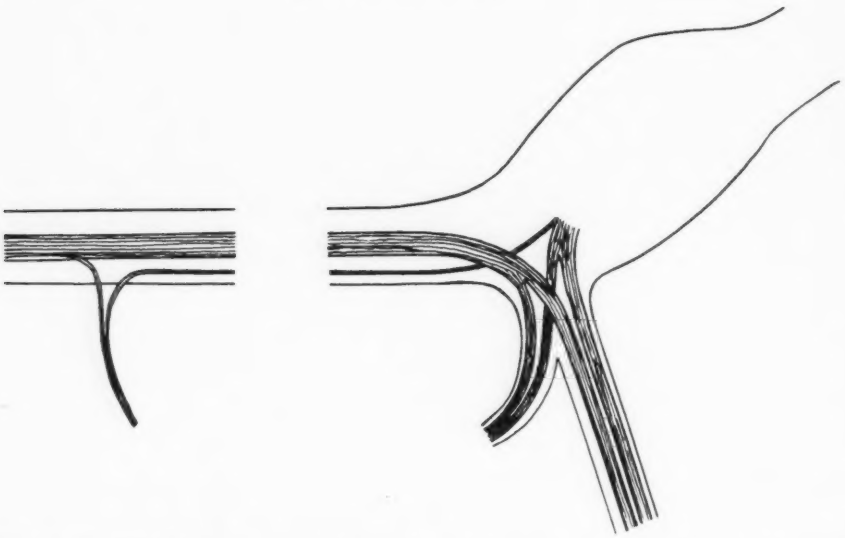

Fig. 17. Diagram showing the composition of the nerves to the auricle and to the branchial heat of fibres from the central nervous system and from the cardiac ganglion. Cf. Fig. 16.

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few are distinctive on account of their much larger size; they all pass into the nerve of the auricle and none into that of the branchial heart. Another particular feature of the auricle nerve is the presence of ganglion cells in it. Some of them are situated near the cardiac ganglion, others singly or in rows of few elements are scattered along the nerve. Morphologically they are quite similar in kind to the cells of smaller size of the cardiac ganglion.

As it approaches the auricle the nerve gives off branches passing on to its ventral and dorsal sides; the thickest of these run near the entrance of the efferent vessels more or less transversely in respect to the axis of the auricle. Some of their branches turn towards the gill spreading out on its efferent vessels near their entrance into the auricle (there are about ten of these vessels), and on the neighbouring parts of the gill. Most of the branches take the opposite course i.e. in the direction toward the ventricle. Anastomosing with one another they form a plexus with thicker bundles running in a general longitudinal direction. (Figs. 16, 18—20.)

Nerves from the visceral trunk.—The number of nerves running from the visceral trunk to the auricle seems to be variable and the character of the thinner ones is often doubtful, but at least one nerve appears to be a constant element of the innervation of the auricle (Fig. 16). Its composition of two sorts of fibres has been frequently ascertained. It was in fact the observation of this nerve which afforded the first evidence of the twofold origin of fibres in the auricle nerves (Fig. 17). On the surface of the auricle the branches of the nerve springing from

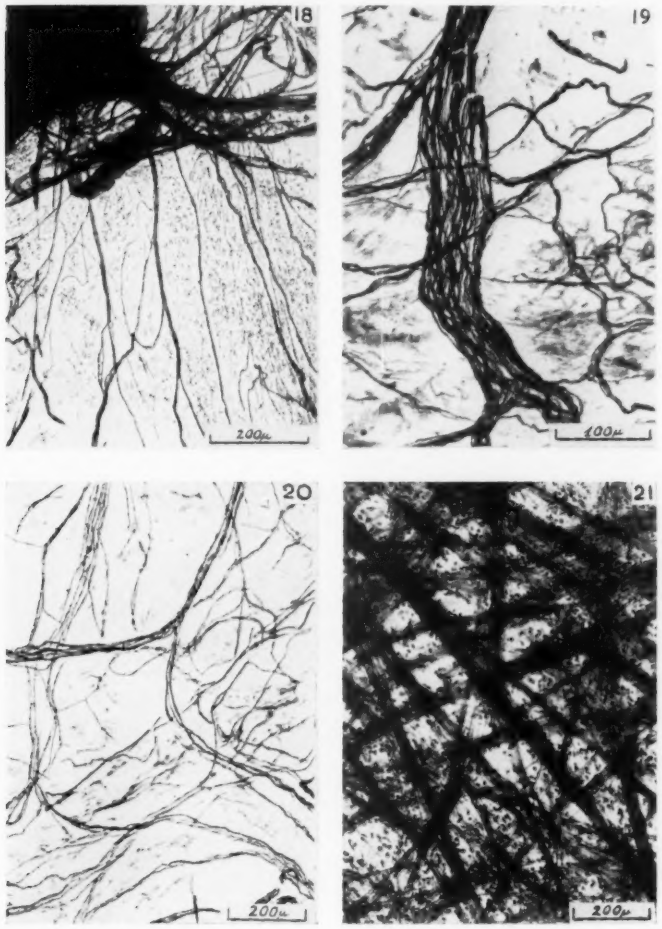

Fig. 18. Part of the cardiac ganglion lying on the flattened wall of the auricle. The thicker fibres running in the upper right part of the figure are nerves of the branchial heart. All other fibres seen in this photograph innervate the auricle.

Figs. 19, 20. Nerves on the wall of the auricle.

Fig. 21. Muscles of the auricle wall fixed in the distended state.
Fig. 18—20, Schultze's method. Fig. 21, Bodian's method.

the visceral trunk mingle in the common plexus with those coming from the cardiac ganglion.

The character of the thin nerves which run from the pericardium with small vessels mentioned above is doubtful. Account must be taken of the fact that in cephalopods the blood vessels and even their capillaries are supplied by a special system of nerves (ALEXANDROWICZ 1928). If therefore a nerve is seen running

along an artery and, as often happens, the distribution of its branches is not clearly visible, it may be difficult to decide whether it is a nerve of the blood vessels or of the organ supplied by the latter. In the case under discussion, if even these dubious elements were taking part in the innervation of the auricle, their contribution, on account of their size, could not be great. On the other hand however such additional nerves might be the cause of some inconsistent results in physiological experiments.

The total number of fibres carried by the nerves to the auricle is much more difficult to estimate than in the ventricle. The counting of fibres can be performed with a certain degree of accuracy only on good transverse sections of the nerve and if the number of nerves to an organ is variable and, moreover, they have a tortuous course, counting is almost impossible. I tried to orientate the ganglion and its branches in a suitable position before fixation, but the nerves suffered from these manipulations and their cross-sections were blurred. Anyhow, the richness of this innervation, especially considering the thinness of the wall of the auricle, is even more impressive than in the ventricle. The thicker bundles of the superficial plexus are made up of dozens of fibres (Figs. 19, 25), and even those approaching the mesial part of the auricle contain many fibres. None of their branches however appear to continue into the ventricle.

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The branches springing from the thicker bundles spread out in all directions distributing their subdivisions to the muscle bundles. The photographs (Figs. 18—20) give an approximative idea of this innervation, but it must be realized that the actual number of nerves is much higher. The distribution of finer elements follows a similar pattern as in the ventricle, and such pictures as in Fig. 14 could be made from the auricle as well.

The fibres are generally of small calibre, but there are also some of conspicuous size (Fig. 26). The latter seem to be few in number, about six in all, but usually two or one only can be seen in one preparation. Thanks to the transparency of the wall they can be traced for a long distances, and it appears that one fibre of this sort sends branches over large areas, perhaps even over the whole auricle. They show also a certain independence of other fibres, for their branches may be seen leaving the bundles in which they associate with thin fibres and following a course of their own. The finest ramifications mix with others and cannot be distinguished from them.

Ganglion cells

Ganglion cells in the wall of the auricle have been found in its lateral third part only. Their number is not less than 50 in each auricle, most of them situated in the vicinity of the entrance of the efferent branchial vessels (Figs. 16, 22). All the cells appear to be of the same unipolar type (Fig. 23), but may very markedly in size (Figs. 24, 25, 27). In appearance and staining reactions they are similar

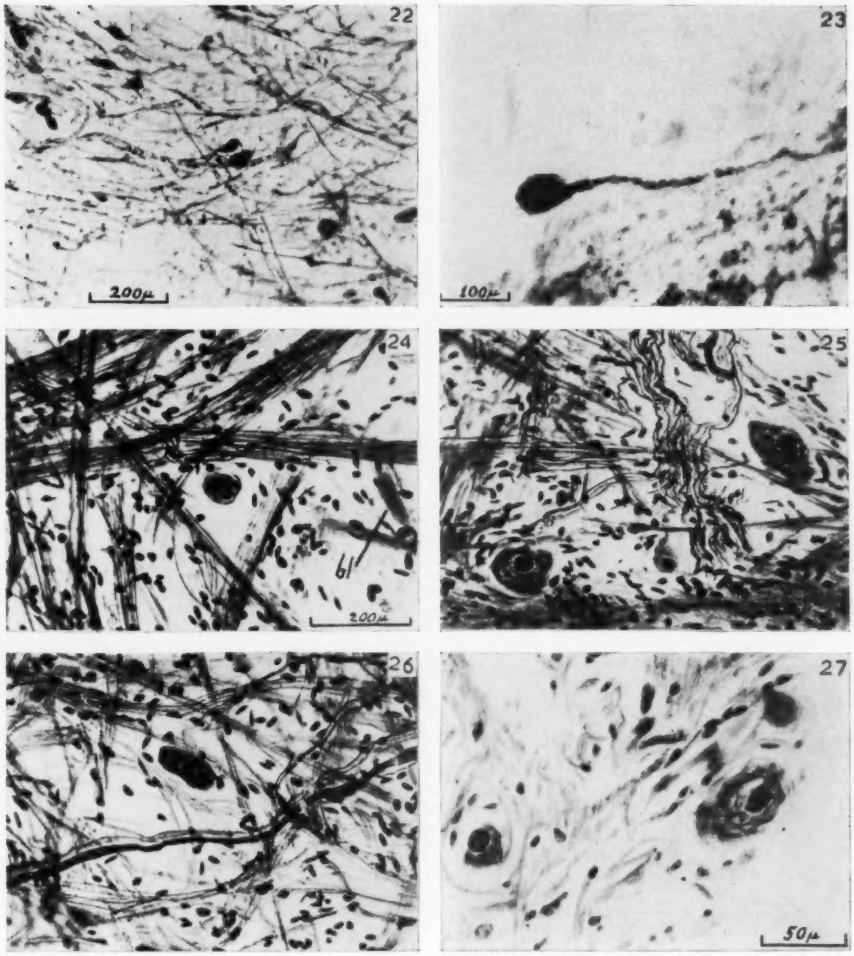

Fig. 22. Several ganglion cells in the lateral part of the auricle. Methylene blue staining.

Fig. 23. Ganglion cell in the auricle. Methylene blue staining.

Fig. 24. Medium sized ganglion cell in the auricle. Note the arrangement of muscle bundles. bl., blood vessel.

Fig. 25. Two large ganglion cells in the auricle showing differently staining structures in their cytoplasm. Lower centre, small ganglion cell situated near a nerve trunk containing many fibres. Same magnification as Fig. 24.

Fig. 26. Nerve on the wall of the auricle with one fibre much thicker than the others. Two ganglion cells seen in the middle partly overlay one another. Same magnification as Fig. 24.

Fig. 27. Two ganglion cells in the auricle. Note the fibrillar structures in the larger cell. Figs. 24—27 from preparations stained with Bodian's method.

to those in the cardiac ganglion. In their cytoplasm two substances staining in different hues can be distinguished and sometimes a network of fine fibrils may be seen (Fig. 27). Unfortunately, with neither of methods used could a good staining of their axons be obtained: with methylene blue only exceptionally a small part of it could be made visible (Fig. 23); in Bodian preparations the axons were too pale to be traced and therefore so much only can be said about their course that they join the nerve bundles in their vicinity. The inference seems to be that their branches spread out with other fibres in the muscles of the auricle.

INNERVATION OF THE BRANCHIAL HEARTS

Structure of the branchial heart

The branchial heart has a very thick wall and a narrow cavity. The wall is of a spongy consistence being pervaded by a system of tortuous canals communicating with the central cavity. It is made up of muscle fibres arranged in a three-dimensional network and polyhedral cells, evidently of glandular nature, filling up the meshes of this network. The latter is much denser than it appears to be on superficial examination, for a great number of muscle fibres uniting the thicker trabeculae are so thin that they can be easily taken for connective tissue fibres or can be passed over unnoticed. Their cross-striation however reveals their true nature.

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Nerves

Of the two nerves emerging together from the cardiac ganglion that to the branchial heart contains many more fibres. After separating from the auricular nerve it splits into several branches and as those are joined by thin nerves springing independently from the cardiac ganglion, a whole bunch of nerves is seen running in the same direction (Figs. 16, 29, 30); some of them are destined for the large veins entering the branchial heart, others ramify on the bulbous enlargement of the afferent branchial vessel, but the majority proceed to the branchial heart itself. On arriving at it the nerves form a plexus with wide meshes (Fig. 31) which is situated on the periphery of the branchial heart, but is covered by a thin layer of its tissue. The branches deriving from this plexus penetrate the wall through its whole thickness following the network of muscle fibres. The same richness of fibres supplying the muscles as in the ventricle and in the auricle can be seen. It was only on the finest muscle fibres that the nerve elements could not be distinguished, but this is probably due to their extreme thinness. Whether the glandular cells have some share in the innervation is an open question and considering the close topographical relations between these cells and the muscle fibres it will not be easy to find the answer.

It will be noted that the valves at the entrance of the veinous trunk which are

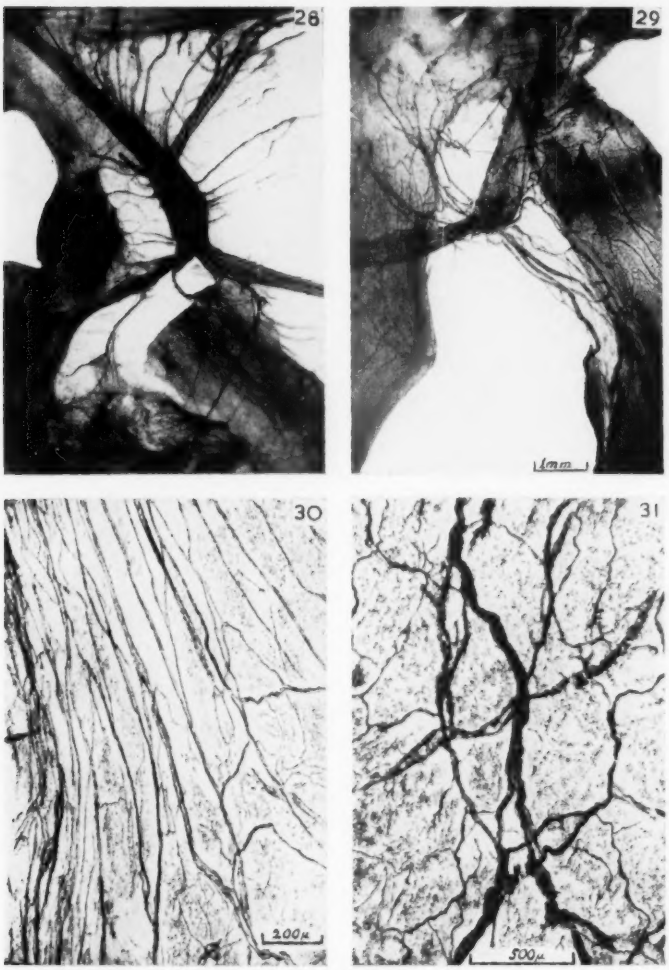

Fig. 28. Left cardiac ganglion with its branches. Dorsal view. Lower right, the auricle with its lateral part cut away. Left, the afferent branchial vessel and a part of the branchial heart. Upper, the branchial ganglionie trunk and the nerves to the ill. Methylene blue staining.

Fig. 29. Left cardiac ganglion with the nerves to the auricle on the left and those to the branchial ganglion on the right. The bulb of the afferent branchial vessel has been slit and flattened with its inner side down. The origin of main auricular nerve does not show.

Fig. 30. Nerves running on the wall of the afferent vessel (upper half of the figure), and of the branchial heart (lower half).

Fig. 31. Nerve plexus in the branchial heart.
Figs. 29—31 from preparations stained with Schultze's method.

almost entirely made up of muscle tissue, receive innervation from the branches of the nerves of the branchial heart.

Some of the thicker trunks of the peripheral plexus pass on to the pericardial gland. Their branches penetrate into the folds of this organ presumably to supply fine muscle fibres situated in the lamina propria of these folds.

As shown in Fig. 17, the nerve of the branchial heart, like that of the auricle, carries two sets of fibres. They are more difficult to trace than those to the auricular nerve because they run deeper in the trunk where they mingle with others coming from the cardiac ganglion.

The fibres in the nerve to the branchial heart are almost all of small calibre. Only four or five are a little thicker, but none of them so thick as those in the nerve to the auricle. There is thus a marked difference in the composition of these two nerves; to this question we shall return below.

No ganglion cells in the branchial heart could be found. On this point the same remarks can be repeated as those made before regarding the occurrence of ganglion cells in the ventricle, viz, that if in an organ of such size there were only a few scattered nerve cells it would not be easy to find them; on the other hand, were they present in appreciable numbers it appears unlikely that they could be missed. The balance of evidence indicates that the neurons of the branchial heart, like those of the ventricle, have their cell bodies concentrated in the central nervous system and in the cardiac ganglion. Consistent with this view is the observation that the ganglion cells occurring in the common trunk evidently belong to the system innervating the auricle since from the point of bifurcation of the trunk they are seen in the auricle nerve but not in that of the branchial heart. In rare instances single cells could be noticed in the latter nerve also, but they were situated near the bifurcation only and can be regarded as the elements of the auricle shifted a little aside from their normal location.

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SOME OBSERVATIONS ON THE INNERVATION OF THE BRANCHIAE

The branchiae have a complicated innervation which requires a special study; some observations however made during the present work which confirm and supplement the data of previous writers may be not out of place. They can be summarized as follows. The ganglionic branchial trunk running in the axis of the gill between its afferent and efferent vessels and surrounded by fibres of the retractor muscle contains numerous cells in its whole length up to the apex of the gill; it is in fact a very elongated ganglion. From it spring nerves like boughs of a tree having a single straight stem. They distribute branches in all parts of the gill: the thickest of them supply the retractor muscle; others run along the vessels and penetrate into the branchial laminae and their folds.

There is no sharp delimitation between the elements of the branchial trunk and those of the cardiac ganglion; some of the latter may even be regarded as

belonging to the same system since a portion of the gill at its base receives the nerves from the cardiac ganglion.

The branchiae have also a sensory innervation consisting of numerous bipolar cells situated in the epithelial layer of the branchial folds and sending their axons into the branchial trunk. They are of the same type as the sensory elements in other organs of cephalopods.

GANGLION CARDIACUM

The examination of the origin of the nerves of the hearts in Sepia provides substantial evidence indicating the important role played in this innervation by the cardiac ganglion. It seems therefore appropriate to add some observations about its anatomy.

The cardiac ganglion is situated at the base of the gill dorsal to the afferent and efferent branchial vessels and therefore it is more easily accessible from the dorsal side of the animal. If the dissection is made from the ventral side it is necessary, in order to render the ganglion visible, to pull the auricle and the afferent branchial vessel a little apart from on another. In this position it is represented in Fig. 16.

The ganglion gives rise to several nerves of various thicknesses (Figs. 16, 28). Besides those to the auricle and to the branchial heart described above, there are others emerging at different points and running to the neighbouring organs such as the pericardium, the muscles of the gill, its afferent and efferent vessels, the big veinous trunks and portions of the gill at its base. A comprehensive description of the ganglion has been given by HILLIG (l.c.), but in his figure the ganglion appears somewhat twisted and the finer nerves arising from it are not represented.

The cells of the ganglion are for the most part of rounded or pyriform shape. Their size varies: the largest which are situated near the periphery measure up to 120 μ, the smallest about 15 μ; the majority lie between these dimensions.

As seen in sections (Figs. 32, 33), the central and a part of the dorsal region of the ganglion are occupied by nerve fibres. In the central region they run in all directions and form a network of thinner and finer elements; fibres situated dorsally belong to a stout fasciculus passing from the visceral nerve through the ganglion directly to the branchial trunk (Fig. 33, *fasc.*). In this fasciculus the following elements can be distinguished: (1) several thick fibres, up to 30 μ in diameter, which are the thickest of all running in the visceral nerves; (2) a mass of fibres of various calibre from 15 μ down to c. 2 μ; (3) several bundles containing a great many of fibres of such small calibre that the individual elements are hardly distinguishable; these cannot be anything else than tracts formed by the axons of sensory cells of the gill. Some of these tracts pass through the ganglion and ascend towards the central nervous system, others coursing deeper become

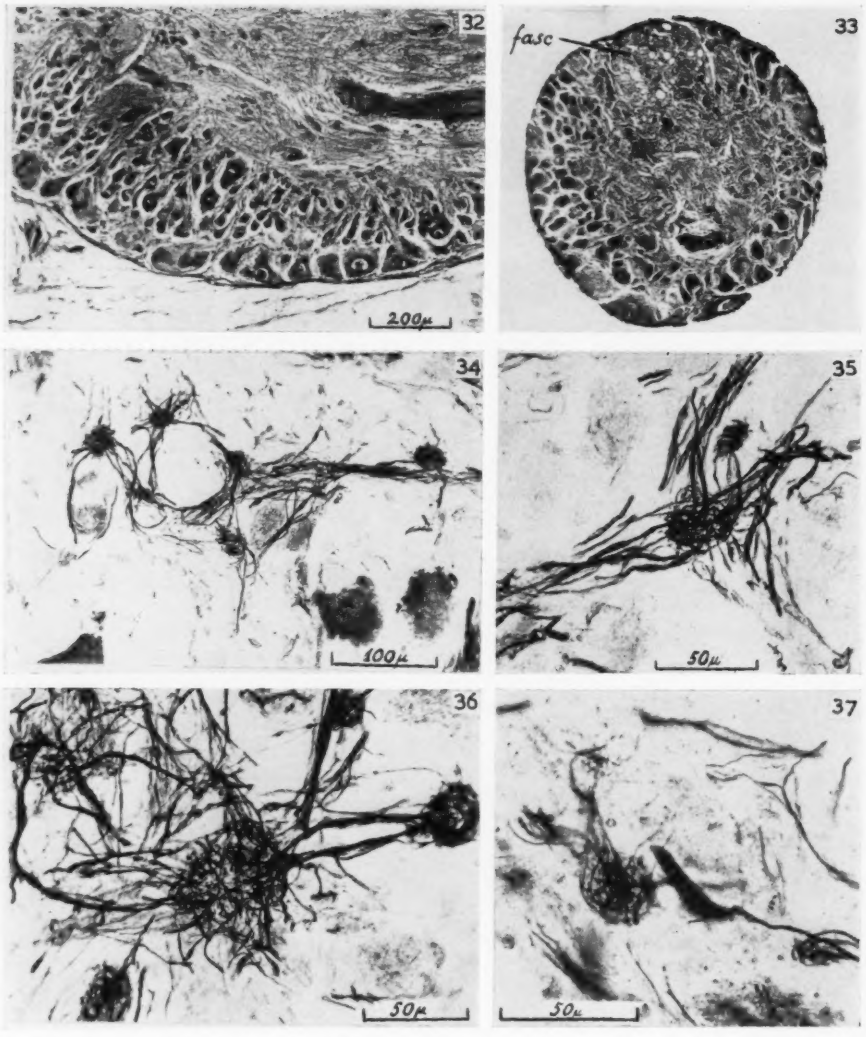

Fig. 32. Longitudinal section through the cardiac ganglion. Note the disposition of the large cells near the periphery. The dark structure seen on the right is the artery passing through the ganglion. Bodian's stain.

Fig. 33. Transverse section through the cardiac ganglion, *fasc.* fasciculus composed of fibres passing through the ganglion to the branchia. Flemming. Iron-haematoxylin.

Figs. 34—37. Glomeruli in the cardiac ganglion. Cajal's method. Note: in Fig. 34 six glomeruli under lower magnification; in Fig. 35 the large number of fibres meeting in one glomerulus; in Fig. 36 the different sizes of glomeruli; in Fig. 37 the spiral winding of fibres within a glomerulus.

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lost in the network of the fibres in the central region of the ganglion and presumably terminate here.

The bulk of the nerve fibres in the ganglion consist of elements of the visceral trunk ending here and of the axons of the ganglion cells. It is obvious that the latter pass into the nerves issuing from the ganglion, but the disposition of neurons assigned for each nerve and the distribution of the fibres of central origin could not be determined. The tracing of individual elements is particularly difficult because of the way the fibres meet in glomeruliform structures formed by coiled fibres (Figs. 34—37). These glomeruli are of various sizes and the number of fibres converging towards each of them appears to vary (Figs. 35, 36). In a suitable position, when a glomerulus is seen from what may be called its pole, a spiral winding of fibres can be observed (Fig. 37). The fibres do not end here, at least not all of them, since some can be seen entering the glomerulus, making a loop in it, and coming out again. The contact between the fibres is evidently brought about by means of fine collaterals.

The glomeruli are located chiefly in the deeper region of the ganglion, near the cells, but sometimes can be found in the zone of the latter. They can be stained selectively with Cajal's method; in preparations stained after Bodian they can also be seen, but because of the equally good staining of many other fibres, they do not stand out so well.

DISPOSITION OF NEURONS IN THE NERVOUS SYSTEM OF THE HEARTS

The observations recorded in the previous sections dealing with the innervation of the ventricle, the auricles, and the branchial hearts, have shown that in each of these organs the nerves in their peripheral distribution are independent of the two others and that they contain fibres of different origin. In order to give a comprehensive picture of the innervation of all three organs together the arrangement of their nerve elements, as it appears most likely to be according to histological evidence, is represented in a diagram (Fig. 38).

This shows the two sets of neurons supplying the hearts: from the central nervous system (1—6), and from the cardiac ganglion (7—11). It will be observed that the former are carried by the visceral nerves which emerge from the visceral ganglia, but there is no evidence that the cell bodies of all these neurons are located in these ganglia as some of them may come from other parts of the brain; the bundles of fibres running to various organs are each represented by one element, but it should be realized that these bundles may contain neurons of various kinds differing not only in their calibre, but also in their function.

The elements decussating in the commissure (1) may belong to the innervation of the hearts, but as their destination is unknown their course is shown as interrupted. The elements drawn in dotted line (7) are those passing from one

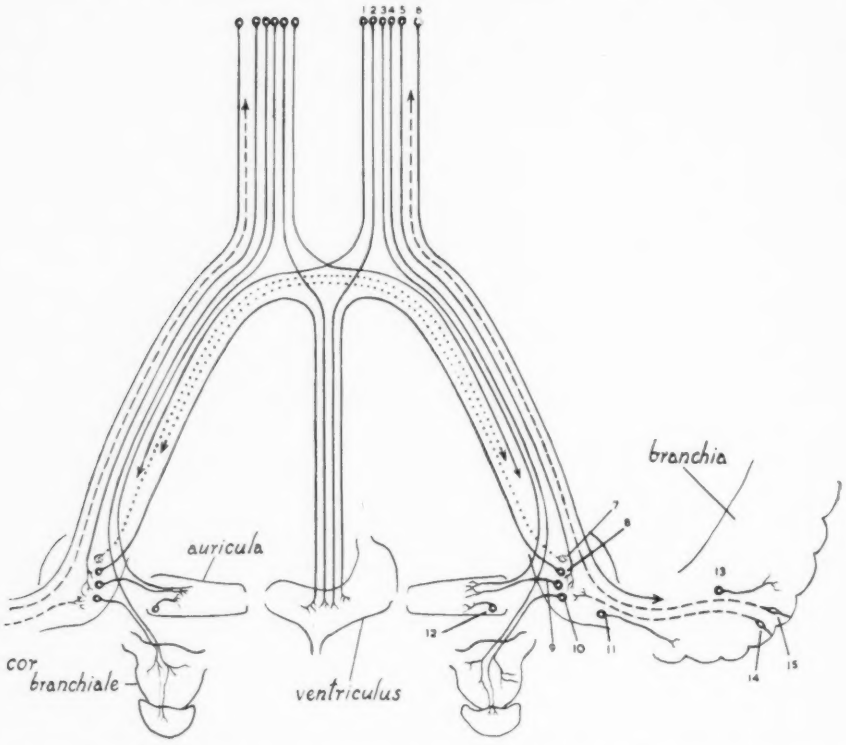

Fig. 38. Diagram showing the disposition of elements of innervation of the hearts and of the branchia in Sepia (partly conjectural). *1—6*, neurons with cells of origin in the central nervous system; *7—11*, neurons with cells of origin in the cardiac ganglion; *12*, neurons in the auricle; *13*, neurons with cells of origin in the branchial trunk; *14*, *15*, sensory neurons of the branchia.

ganglion through the commissure towards that of the opposite side; as mentioned before, their passage through the commissure is not absolutely certain. Moreover, if even this question could be answered in the affirmative, the problem of their final course would remain for they may end either in the hearts or in the ganglion.

Of the elements of the innervation of the branchia the following are shown: neurons with their cells of origin located in the branchial trunk (13) and in the cardiac ganglion (11); sensory neurons which send their axons to the central nervous system and to the cardiac ganglion (14, 15); neurons with their cell bodies located in the central nervous system (6). As the distribution of the latter has not been determined, only the general direction of the fibres running towards the branchia is shown; as a matter of fact there are here fibres of various calibres and presumably having different functions; it is also probable that they terminate not only in the muscles of the branchia, but also in the ganglionic trunk.

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The elements of the innervation of the big veins which by their rhytmic contractions contribute to the propulsion of the blood, are not included in the diagram. These veins are supplied by nerves arising from the cardiac ganglion and from the visceral trunks, but the problem of the location of neurons making up these nerves requires further study.

Discussion

It is understandable that when starting the investigations of a molluscan heart I was bound to have, on the basis of comparative anatomical considerations, certain ideas as to all the conceivable features of this innervation which might be encountered. During present work however, more than once facts came to view which could hardly have been expected and which were altogether surprising.

One of them was the extraordinary high number of nerve units involved in the innervation of cephalopod hearts. As stated before, the ventricle alone receives more than 800 fibres. The sum of all those running to the two auricles and to the two branchial hearts is certainly not less than that. This estimate is not exact, but it is doubtless on the lower side and anyhow if it can be assumed that the total number of neurons supplying the hearts in Sepia is well over one and a half thousand this figure is impressive enough and it is many times higher than in the hearts of some other invertebrates. In crustaceans, for instance, the local system of the heart, i.e. the system of neurons with their cell bodies located in the heart, contains a very limited number of elements, viz 9 in Decapoda (with exception of Astacus astacus which has 16), 14 in Stomatopoda, 6 in Isopoda, Amphipoda and Mysidacea (Alexandrowicz 1932, 1934, 1952, 1954, 1955). A few fibres link the local system with the central nervous system and thus the total number of neurons supplying the heart, although it has not been exactly estimated because of the occurrence of some fine fibres which are difficult to count, is not high, in the Decapoda it appears to be less than thirty.

In insects the figures according to the few data available, are a little higher. In *Blatta* (*Periplaneta*) *orientalis* the local system contains about 40 neurons (Alexandrowicz 1926), in *Carausius morosus* probably the same number (Opoczyńska-Sembratowa 1936), in *Periplaneta americana* about 32 (McIndoo 1945), in *Schistocerca gregaria* about 40 (unpublished observation of the writer). The number of fibres running from the central nervous system to the heart has not been determined in either case, but as the nerve branches which could carry them are very thin, these fibres cannot be numerous. More nerve units supply the heart of *Limulus*. No exact data could be found, but its ganglion cells of small type are said to be numerous (Nukada 1925). Also in scorpions the nerve trunk running on the dorsal side of the heart contains a conspicuous number of

ganglion cells (unpublished observation of the writer). However in these cases too the number of neurons seems to be inferior to that found in Sepia.

It is true that in Crustacea there are other nerve elements having certain relations with the action of the heart such as the nerves of the arterial valves, of the pericardium, of the pericardial organs, but all these systems belong to organs which with exception of the arterial valves have no counterparts in Cephalopoda. Two organs only are truly comparable, namely the ventricle of a cephalopod and the heart of a crustacean, both having the identical function of driving the blood into the arterial system of the body. In animals like e.g. Sepia and Homarus, the amount of muscle tissue in these organs, in specimens of approximately the same weight, would not differ very much, but the number of neurons supplying them will be in Sepia about thirty times greater.

It is only the high figure of this ratio that is surprising. The fact itself that more nerve units would be present in cephalopods than in arthropods was to be expected for in other organs which are comparable, as e.g. the digestive tract, the number of nerve elements is strikingly high (ALEXANDROWICZ 1928), and in general there appears to be everywhere in cephalopods a profusion of nerve elements, one might say, an uneconomical use of nervous substance compared with crustaceans and insects. It appears that for performing analogous functions the latter employ much fewer neurons which have evidently developed a higher degree of efficiency.

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In spite of the abundance of its nerves, the ventricle of Sepia, when taken out of the animal body, is in a certain sense poorer in nerve elements than the crustacean heart in similar conditions for in the former all its neurons have been mutilated and all the cells eliminated from the innervation, whilst in a crustacean heart only the neurons of central origin have been severed, with all others, i.e. of the local system, remaining completely intact. This ought to be taken into account when comparing the behaviour of these organs in physiological experiments.

In view of the abundance of nerve elements in the organs of Sepia the more unexpected was the absence of ganglion cells in the ventricle. It became obvious that the cephalopod heart differs on this point from that of arthropods. It is conceivable however that this difference is not as fundamental is it seems to be at first sight. Considering the fact that in both cases the heart is supplied by two sets of neurons, one of them coming from the central nervous system, it may be conjectured that the second set of neurons, i.e. originating in Sepia from the cardiac ganglion, might be the counterpart of the local system in arthropods with the difference that its cell bodies are located outside the heart (which does not imply that these systems must have an identical function). It is conceivable that such a disposition might have been necessitated by the complexity of the mechanism of blood circulation in cephalopods since all the organs driving the blood must pulsate in a definite sequence and as in this coordination the nervous system

presumably plays an important role, the concentration of its cells in one ganglion appears to be an appropriate arrangement.

According to this assumption there is no need for nerve cells to be present in either of the organs investigated. It was therefore surprising to find them in the auricle. As to the character of the latter two possibilities have to be considered; either these cells are of some special kind and have another function than those situated in the cardiac ganglion, or they all belong to the same system with some of its cells located not in the ganglion but in the auricle. I am inclined more to give credit to the first alternative not only because it is difficult to imagine what the different function of the cells in the auricle could be, but also because of the presence of nerve cells along the auricular nerve. These cells, constituting a link between the elements of the ganglion and those in the auricle, can be regarded as being disseminated along the path of the concentration of nerve cells which had not been completely achieved.

This conception however does not give the answer to the question why some nerve cells should remain in the auricle as its constant components. If our assumption that they are absent elsewhere is correct then there must be some reason for their presence in one of the pulsating organs only. May be, in physiological experiments some differences in the behaviour of the auricle compared with that of the auricle and of the branchial heart could be noticed which might throw some light on this problem.

As regards fibres running to the hearts from the central nervous system some of their features are worth pointing out. As stated above, among the elements carried by the visceral trunks there is one set of fibres running without interruption to the hearts and another set terminating in the cardiac ganglion. Although a clear picture of the connections of the latter could not be obtained there can be little doubt that these fibres enter into synaptic relations with the cells of the ganglion which, as we know, send their axons to the hearts. It follows therefore that the impulses from the central nervous system to the pulsating organs can be mediated (a) by a direct one-neuron way, and (b) by way of two neurons, the first of them relaying the impulse in the cardiac ganglion to the second neuron which conveys it to these organs (Fig. 38).

Another feature which has to be taken into account in physiological considerations is the presence of the thicker and thinner fibres in the nerves supplying the hearts. It is difficult to draw a line separating them into two distinctly delimited groups since many fibres, especially in the cardiac nerve, show gradations in their diameters; but allowing for dubious cases it can be stated first, that the thick fibres are much less numerous, and, secondly, that their number in proportion to the thinner ones is not the same in the nerves, viz, it is much greater in the nerve to the ventricle that in that to the auricle, and in the nerve to the branchial heart none of fibres is of such calibre as in the two other nerves. From this the inference may be drawn that the impulses propagated by the thick fibres are

unevenly distributed to the three organs in question and it would be interesting to see what differences in reactions of these organs could be attributed to the unequal composition of their nerves.

It should be mentioned that KRUTA (1936), on the basis of experiments on excitability of the visceral nerves in Sepia came to the conclusion that they carry fibres of various sorts. The difference in their calibre have also been observed (ARVANITAKI, FÉSSARD & KRUTA, 1936).

It remains to draw attention to some observations on the innervation of the branchia which presumably have an important bearing upon the problem of the regulation of the circulation of the blood in cephalopods. The presence of the sensory elements on the surface of the gills makes it evident that one of the organs of the circulatory mechanism is adapted for receiving stimuli from its environment, presumably responding to variations of pressure or changes in the composition of the water in the mantle cavity. Histological evidence indicates that tracts made up of axons of these sensory elements run to the central nervous system and to the cardiac ganglion. These therefore must be the ways through which are transmitted the impulses influencing the respiratory mechanism and the action of the hearts.

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Physiological experiments concerning the function of the heart nerves in Cephalopoda have been made by several writers (bibliography in BAUER (1909), BRÜCKE (1925), KRIJGSMAN & DIVARIS 1955). They have shown, as their main result, that in the visceral nerves there are fibres regulating the action of the hearts: the presence of inhibitory nerves was established by BERT (1867) and confirmed by later experimenters; as to the occurrence of accelerator fibres discovered by LÉON FREDERICQ (1878) some doubts were put forward (RANSOM 1884, FRY 1909), but the experimental work of CARLSON on Loligo (1909), of HENRI FREDERICQ & BACQ on Octopus and Eledone (1939), and of KRUTA on Sepia (1936), confirmed Léon Fredericq's conception. There is therefore adequate evidence indicating that of the two systems of the neurons supplying the cephalopod hearts that of central origin transmits inhibitory and excitatory impulses. Whether some other impulses are mediated by the same way is an open question.

There remain still several hundred neurons making up the second system, i.e. originating from the cardiac ganglion, whose role has to be determined. Only few investigations are known to have been made which have some bearing on this problem. RANSOM (1884) who made a series of experiments with Octopus cutting through the nerves of the hearts at various points, came to the conclusion that the "cardiac" system which he also calls "intrinsic" or "intra-cardiae" system, is "a coordinating mechanism between the systemic and the branchial hearts", and that "the oldest and central organ of the cardiac apparatus is the ventricle, to the service of which the other parts are adapted". Ransom has also observed the action of impulses coming from the gills; later CARLSON (1909) working with

Loligo and Octopus confirmed Ransom's view regarding this action namely that through the cardiac ganglia pass the inhibitory reflexes from the gills to the branchial hearts.

No reference concerning the function of this system in Sepia could be found. Some of Ransom's conclusions may prove to hold good for cephalopods in general, but the results of his experiments with Octopus cannot be regarded as providing adequate evidence of the function of the heart nerves in Sepia in view of the important differences in the arrangement of these nerves in decapods. Besides, Ransom's experiments were performed without knowledge of the finer anatomy of the nervous system of the heart. This subject in octopods requires more exact study and only after it is better known would it be possible to assess the validity of Ransom's assertions concerning the presence of nervous connections, other than through the main trunks, between the ventricle and the branchial hearts.

The disposition of the heart nerves in Sepia which the above findings show to be the most likely does not tally with Ransom's theory about the dominance of the ventricle over the action of other pulsating organs; one would rather presume, as has been alluded to before, that the gills may play a more important role. If however it could be shown that also in Sepia the impulses from the ventricle pass to the branchial hearts, as is assumed by Ransom for Octopus, then it would be necessary to admit that either some elements have been overlooked—such a possibility can never be ruled out—or the character of the elements observed have been wrongly interpreted.

All these considerations make it clear that for a better understanding of the function of the mechanism of circulatory organs in Cephalopoda more research work both on their morphology and physiology is necessary: on the one hand the disposition of nerve elements in octopods and in other species of decapods should be made better known, on the other hand the results of observations on the innervation of the hearts in Sepia must pass the test of physiological experiments which would verify the assumptions and corroborate or invalidate the conjectures.

Summary

1. In the innervation of the hearts (ventricle, auricles and branchial hearts) of *Sepia officinalis* two systems of neurons take part: one originating in the central nervous system, the other in the cardiac ganglion (*syn.* ganglion of the branchial heart, 2nd heart ganglion, branchial ganglion). The total number of these neurons is estimated to exceed 1600.

2. The ventricle receives its nerve supply through the cardiac nerve arising from the commissure of the visceral nerves. The fibres of the cardiac nerve derive

partly from the visceral ganglia of the brain and partly from the cardiac ganglion; the latter ascend in the visceral nerve to the commissure. The cardiac nerve pierces the wall of the ventricle to appear on its inner surface as a stout trunk from which arise branches running in all directions: the thickest of them spread in the innermost layer of the muscles and give off branches penetrating into the heart wall and supplying all its muscle fibres with their abundant subdivisions. There is no communication between this system of nerves and those of the auricles or of the arteries arising from the ventricle. Ganglion cells in the ventricle have not been found.

3. The main nerve to the auricle arises from the cardiac ganglion together with the nerve to the branchial heart; a smaller nerve branches from the visceral trunk. Each of these nerves carries fibres of the two systems of neurons, i.e. coming from the brain and from the cardiac ganglion. On the wall of the auricle the nerves form a plexus giving off branches ramifying on the muscles. Some additional nerves of finer calibre may spring from the ganglion and from the visceral nerve and join the plexus. Ganglion cells numbering no less than 50 are scattered in the lateral part of each auricle near the entrance of the efferent branchial vessels. Their axons pass into the bundles of the plexus.

4. One stout nerve and several thin ones run from the cardiac ganglion to the branchial heart. The stout nerve associating at its root with that of the auricle contains also fibres of the two systems mentioned above. On the wall of the branchial heart the nerves form a plexus giving off branches which follow the trabeculae of the network of muscle bundles extending through the whole thickness of the wall of this organ. No ganglion cells in the branchial heart have been found.

Some of the trunks of the nerve plexus of the branchial heart pass on to the pericardial gland and ramify in its folds.

5. The cardiac ganglion contains cells of various sizes, the largest of them situated near the periphery. Some of the fibres of central origin carried to the ganglion by the visceral nerve terminate in the ganglion, while the others pass into the ganglionic trunk of the branchia. Peculiar features of the cardiac ganglion are glomerular structures formed by coiling fibres which evidently enter into synaptic relations within these glomeruli.

6. The gills, in addition to the nerves coming from the central nervous system and those arising from their ganglionic trunks, have also a sensory innervation consisting of numerous bipolar cells situated at the surface of the branchial folds.

7. As regards the function of elements of the nervous system investigated the following features of this system may provide some indicative evidence.

The number of fibres running to the hearts from the cardiac ganglion appears to show that the impulses transmitted by them to the muscles of the hearts play an important functional role. The concentration of the nerve cells of this system

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in one ganglion may facilitate is presumable function of coordinating the action of various pulsating organs.

From the course of the fibres of central origin it can be deduced that the impulses from the central nervous system can be transmitted to the hearts either by a direct path consisting of one neuron, or after being relayed to a second neuron in the cardiac ganglion.

From the difference of the calibre of the fibres of central origin it can be inferred that these fibres differ in their function also.

The occurrence of the sensory innervation of the branchiae indicates that they receive stimuli from their environment which presumably elicit reflexes adjusting the mechanism of respiration and of the blood circulation.

Appendix

HINTS ON DISSECTION

For the study of the general anatomy of Sepia large specimens are recommended, but those of smaller size are much to be preferred if one's aim is to obtain whole mounted preparations of the nervous system of the hearts.

For narcotizing chloroform can be used. The animals should be opened from the ventral side and the inksac must be taken away. Then all those parts of the viscera which can be easily removed without injuring the hearts and the visceral nerves should be cut away; the more accurate cleaning can be done later. When sectioning the renal appendages care must be taken not to damage the cardiac nerve. The remaining organs can now be taken out in one piece: in order to do this, the membrane covering the liver should be cut transversely at some distance in front of the anus; with lateral incisions a strip of this membrane containing the vena cephalica and the visceral trunks can be separated and pulled backwards; the oesophagus and the anterior aorta which become thus exposed should be cut through; prolonging the lateral incisions backwards and outwards and cutting through the tissues between the viscera and the body wall the whole mass containing the heart, the gills (parts of which can be sectioned), the visceral nerves with adhering membranes and parts of other organs can be taken out and pinned down to a paraffin plate either with the dorsal or the ventral side upwards. Each of these positions has some advantages for further dissection: thus the cardiac ganglion is more easily accessible from the dorsal side and the cardiac nerve from the ventral side, whilst the hearts can and should be examined from both sides. The aim of further dissection is to obtain preparations with the visceral nerves, their commissure and the cardiac ganglion well exposed and remaining in connection with the hearts and the branchiae. All unnecessary parts must therefore be removed as far as possible and after this has been done the preparation can

be stained, fixed etc. and mounted in toto, or some portions of it may be cut out and treated separately. In all cases it is advisable to keep the organs attached to the paraffine plates as long as is necessary for preventing the structures from altering their shape and position. If some structures should be deformed when transferred from alcohol into xylol, the paraffine plates can be replaced by polyethylene plates. The latter can be used from the beginning, but as during dissection many pins have to be put in and pulled out, this is more smoothly done when paraffine plates are used.

For the study of the nerves in the ventricle its dorsal or ventral wall should be cut from auricle to auricle, keeping the line of incision nearer the posterior margin of the ventricle and extending it to the anterior aorta. The auricles, or parts of them, can be left with the ventricle which now should be attached to the paraffine plate in a well stretched state with its inner surface up. In the auricle the nerves can be observed from either side and also without opening the wall. In the latter case they even stain better with Schultze's method.

The branchial heart should be opened and pinned down with the inner or outer surface upward; the latter position is more suitable for studying the large trunks reaching this organ.

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STAINING METHODS

Methylene blue.—Methylene blue was applied in two ways: as a weak solution in which the tissues were immerged, or in a stronger concentration for injection. In the former (10—15 drops of 0.5 % solution of methylene blue in dist. water added to 100 c.c. of sea water) the organs were left for from 5 to 20 hours. The stronger solution (10 drops of methylene blue solution to 10 c.c. of sea water) was injected into the region of the commissure, near the cardiac ganglion, along the visceral nerves and around the auricles with the organs left in situ, or after they have been taken out from the animal body. The injected preparations can be kept in the moist chamber or immersed in a weaker solution of methylene blue and the staining by immersion and injection can be combined in various ways. The injected fluid does not spread in the connective tissue, but produces a local blister, and the methylene blue in such concentration in sea water can form precipitates which have to be removed later, but despite this inconvenience this method is helpful in showing deeper lying elements. Whichever method is uesd it is necessary, as staining proceeds, to dissect away the tissues hindering the access of the dye to the nerves, to remove the precipitates and to correct the position of the organs by fixing them with small pins in such a way that the nerves are well exposed. This can be a tedious job, but a good deal of success depends on it. After staining, the preparations have to be treated in the usual way, i.e. fixed in ammonium molybdate solution, washed in water, dehydrated in alcohol, cleared in xylol and mounted in xylol-dammar.

With methylene blue the following nerve elements can be stained: the visceral nerves with their commissure, the cardiac nerve, the cardiac ganglion and the nerves arising from it, the trunk in the gill and its main branches, the sensory cells of the gill and the nerves of the blood vessels. Difficult to stain are the ganglion cells in the auricle and in the commissure. For showing the nerves in the hearts methylene blue is useless, not only because these nerves do not stain well, but also because the muscle fibres and often the connective tissue fibres stain readily and their arrangement is such that the distinguishing of nerves is difficult. Paradoxically, very fine fibres are sometimes better discernible than the large trunks. Suffice it to say that with methylene blue preparations I was unable to decide whether structures in the ventricle which proved to be its main nervous trunks were of nervous nature or not.

Methylene blue can render good service during dissections, since even a faint staining makes the outlines of the structures much more distinct. Therefore, in many instances, preparations intended for treatment by other methods were first put in methylene blue solution. It is of course better not to expose the tissues too long to the action of sea water, but it was found that even after they had been kept in methylene blue solution for 10 hours they could be used for silver impregnation of the nerves with Schultze's method.

Silver methods.—Schultze's modification of Cajal's method (v. Romeis 1948) was the chief method applied. The procedure set forth below is similar to that used before by me for cephalopod eye with two modifications viz, the use of an acidified reducer and the treatment of the preparations by a bleaching agent. The idea of adding a small quantity of nitric acid to the reducer has been borrowed from Palmgren (1948), but for my formula a little more acid is taken as this proved to be useful in diminishing the formation of precipitates. The non nervous tissues however become a little deeper coloured, with a tinge of red, than in preparations treated by the standard reducer. In order to make the background brighter a solution of potassium cyanide has been used, the agent applied for bleaching in the older gold methods. The whole procedure was as follows.

1. Fixation in: formol (neutralised with Na_2CO_3) 15 c.c., sea water 100 c.c., 48 hours or longer.
2. Washing in dist. water, 6—12 hours.
3. Treatment with sodium hydroxide solution: stock solution (NaOH 4 %) 6 c.c. dist. water 100 c.c., 24—48 hours; this was the concentration most frequently used, but with the weaker and stronger ones good results have been obtained.
4. Washing in dist. water changed several times, 24 hours.
5. Impregnation with silver nitrate 3 %, 48—72 hours.

6. Reducing. Stock solution (hydrochinon 2.5 grm., formol 5 c.c., dist. water 95 c.c.) 2—3 c.c.
   dist. water 100 c.c.
   nitric acid 1 % 10 drops
   The process of the reduction must be observed under low power microscope.
7. Washing in dist. water several times changed, 1—2 hours.
8. Toning in 0.1 % gold chloride solution.
9. Fixing in 5 % sodium thiosulphate solution, 10 minutes.
10. Washing in dist. water, 1 hour or longer.
11. Bleaching in 0.5 % potassium cyanate solution in dist. water. The progress of bleaching should be controlled and interrupted as soon as the desired decoloration is obtained. Longer action of cyanide affects the staining of nerve fibres; it appears that the difference between the "impregnation" and the "staining" of nerve tissue with metal salts manifests itself in the degree of resistance to the cyanide.
12. Washing in dist. water, alcohol, etc.

Schultze's method has been very valuable in showing the nerve fibres in all the organs of Sepia investigated, but it proved unsuccessful in staining the nerve cells in the auricle and in the commissure.

The impregnation of nerves is uneven so that the number of fibres in the same trunk may seem to be different in various preparations, and the staining of deeper situated elements is often unsatisfactory. It is for instance difficult to get a good impregnation of the cardiac nerve in its passage through the wall of the ventricle. The organs with thin walls, from smaller animals, are therefore preferable.

There are certain differences in the stainability of the nerves for which no reason could be found. Thus in the ventricle they stained readily, in the branchial heart less well and in the auricle very unevenly and often not at all although these organs were subjected to identical treatment.

Bodian's method was used not only for sections but also for the whole ventricle and for the auricle opened and fixed in an extended state. As fixative Bouin's fluid was used and the staining performed according to the original prescription with the exception of the final differentiation for which instead of oxalic acid anilin alcohol (alcohol 50 % 100 c.c., anilin oil 2 drops) was taken. Anilin alcohol, introduced into the gold technique by Nabias, has been recommended by Palmgren (1948), in the concentration indicated above, as an intensifier of gold toning of preparations stained by his silver method if the organs contain not only nerve elements but non nervous tissues as well. The advantage of anilin alcohol over oxalic acid in leaving the background in a lighter hue proved to hold good for Bodian preparations and thanks to this modification the nerves and other structures of the auricle mounted in toto could be clearly seen. The wall of the

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ventricle is of course too thick for this, but it is possible to obtain quite good preparations of its nerve trunks by separating them from the heart wall. This can be done when the ventricle has passed through all stages of staining procedure and is brought into xylol. The muscles can then be taken away piece by piece starting from the outside. As the muscle tissue becomes brittle in xylol this is easily done, only near the inner surface it is necessary to proceed with care. The innermost bundles of muscles should be left with the nerve trunks.

Bodian's method has the advantage over other silver methods in that it stains the nerve elements more evenly so that for instance the number of fibres in nerve trunks can be much better estimated than in preparations stained with Schultze's method. It is also more universal in that both nerve cells and fibres can be stained with it and it proved to be the best of all methods for demonstrating the ganglion cells in the auricle; their axons however did not show well. Bodian's method has also proved valuable in showing the cross-striation of the muscle fibres very distinctly.

Cajal's method was applied following the procedure recommended for cephalopods by SERENI & YOUNG (1932) with the time of treatment by silver nitrate shortened from 14 to 5—8 days. 1. Fixation in d'Ancona's mixture (chloralhydrate 2.5 grm, pyridin 20 c.c., alcohol 50 % 80 c.c.). 2. Dist. water. 3. Alcohol 96 % 24 hours. 4. Silver nitrate 5 % 5—8 days at 37°. 5. Reducer: (hydrochinon 1 grm, formol 5. c.c., dist. water 95 c.c.). This method was used for the cardiac ganglion and it was found suitable for selective impregnation of the glomerular structures contained in it.

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Über das Zentralnervensystem von *Heterocephalus glaber (Rodentia, Bathyergidae)*

von

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Waldau/Bern (Schweiz)

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Einleitung, Systematik, Fragestellung

Obwohl schon zahlreiche Studien über *Heterocephalus glaber* (Abb. 1) und andere *Bathyergidae* vorliegen, bleibt die systematische Position dieser Familie von Nagern stets kritisch. Manche Autoren sehen die *Bathyergidae* als *Hystricomorpha* an, während andere sie in die Subordnung der *Myomorpha* einreihen. WOOD (1955) gewährt der Familie den Rang einer Subordnung und SIMPSON (1945) führt sie als Familie der Superfamilie *Bathyergoidea* (OSBORN, 1910) an, welche *„Hystricomorpha incertae sedis"* wären. Die in letzter Zeit erschienenen, sehr gediegenen biologischen und vergleichend-anatomischen Untersuchungen von OSMAN HILL und Mitarb. (1957) führen zum Ergebnis, dass ... *„New evidence is presented emphasizing the apartness of the Bathyergoidea from all other rodent groups but no new evidence is forthcoming warranting association with the hystricomorphs or other suborders of existing Rodentia"*...

Für die Biologie, Habitat, Verhalten im Biotop und in Gefangenschaft, Nestbau, etc. des *Heterocephalus glaber* sei hier auf die ausführliche Darstellung von OSMAN HILL und Mitarb. verwiesen. Interessante ethologische Beobachtungen verdanken wir Prof. D. STARCK (1957), die während der von STARCK geleiteten Abessinienexpedition 1955—56 gesammelt wurden.

OSMAN HILL untersucht in seiner Arbeit in den Grundlinien auch die Morphologie des Gehirnes. Wir wollen im folgenden auf seine Befunde Bezug nehmen, die Beschreibung ergänzen und sie unter vergleichend-hirnanatomischen Gesichtspunkten betrachten. Bei der grossen Vielfalt der Hirnformen der Nagetiere halten

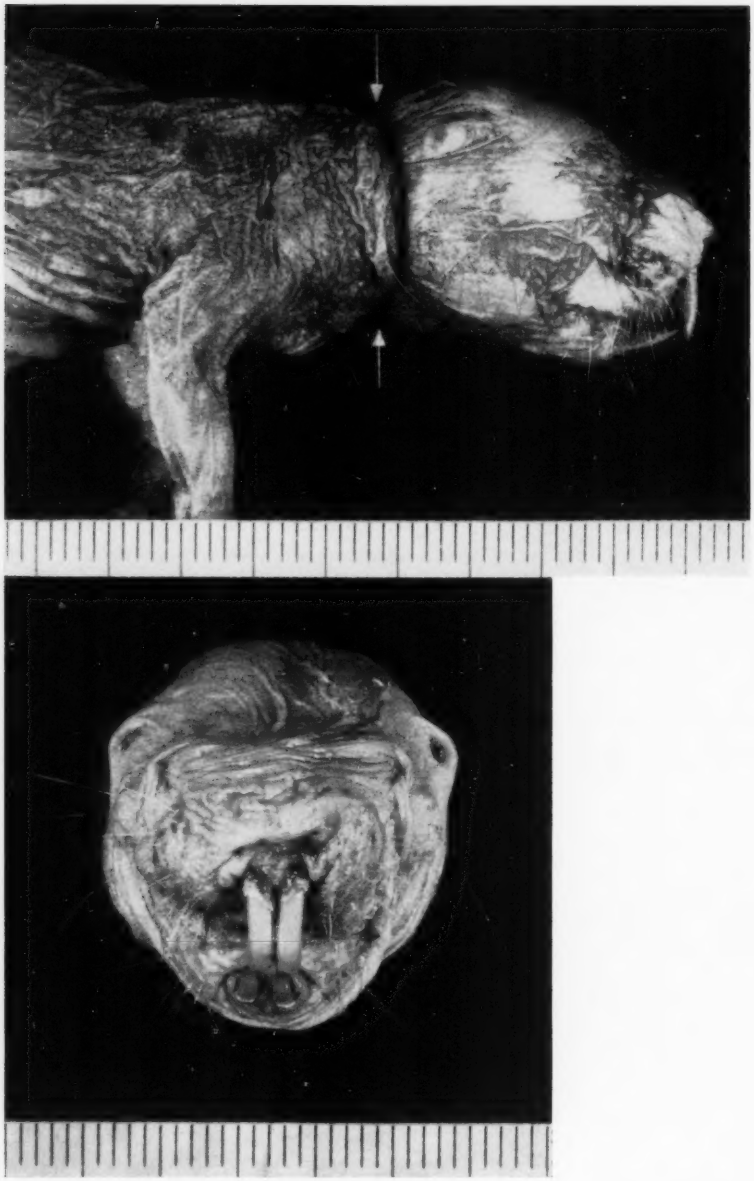

Abb. 1. *Heterocephalus glaber:* Aufnahme von 2 fixierten Präparaten; zwischen den Pfeilen artifizielle Einschnürung (T 93 und T 94 — Sammlung Pilleri).

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wir für möglich, dass eine Beziehung zwischen Hirngestalt und Taxonomie besteht, wobei die Hirnform in manchen Fällen besser als andere Organe auf die natürliche Stellung im System hinweist. Danach ist folgende Untersuchung gerichtet.

Material

Durch das Entgegenkommen des Herrn Dr. W. C. Osman Hill, Prosector der Zoological Society of London konnten wir einige formolfixierte Exemplare von *Heterocephalus glaber* (Patria: Somaliland) erhalten. Dieselben Präparate hatten bereits in seiner Monographie (1957) Verwendung gefunden, auf die wir auch für nähere Angaben über das Material verweisen.

Hirn- und Körpergewicht

Für Kenja-material von *H. glaber* gibt Osman Hill als Extremwerte des Körpergewichtes 20—80 gr. an, wobei hier offenbar Tiere verschiedenen Alters vorliegen. Exemplare aus Somaliland wogen 13—43 gr. Das Hirngewicht ist bei dem uns zugeschickten erwachsenen Exemplar (Somaliland), nach langjähriger Formolfixation 0,52 gr. In Ermangelung der Frischgewichte sind wir bei der Bestimmung des *Cephalisationsgrades* vorläufig auf aproximative Werte angewiesen.

Beschreibung des Zentralnervensystems

Allgemeine Formverhältnisse: Dorsal betrachtet (Abb. 2, 3) sind die stark entwickelten Bulbi olfactorii vom Frontalpol nicht überdeckt und durch eine tiefe Querfurche von diesem getrennt. Der Endhirnmantel ist breiter als lang. Die Sagittalspalte ist etwa 6 mm lang. Die Frontalpole sind stark abgerundet, die seitliche Hemisphärenausladung ist beträchtlich. Caudal klaffen die Mantelkanten, in Form einer breiten Aushöhlung, die einen Teil des Kleinhirns beherbergt, stark auseinander. Die Vierhügelplatte ist zum kleinen Teil dorsal sichtbar. Das Kleinhirn ist wesentlich schmäler als das Grosshirn, an den Seiten gleichmässig abgerundet. Auf der dorso-caudalen Fläche des Kleinhirns findet sich kein Sulcus paramedianus, sodass die Bildung eines „Vermis cerebelli" nach der alten Bezeichnung nicht vorliegt. Caudal überdeckt das Kleinhirn in Form eines Halbmondes einen Teil der Medulla oblongata, die konisch verengt in das Rückenmark übergeht. Bei *lateraler Betrachtung* ist die Fissura rhinalis makroskopisch nicht feststellbar. Mikroskopisch (Abb. 8) ist die Grenze zwischen Palaeo- und

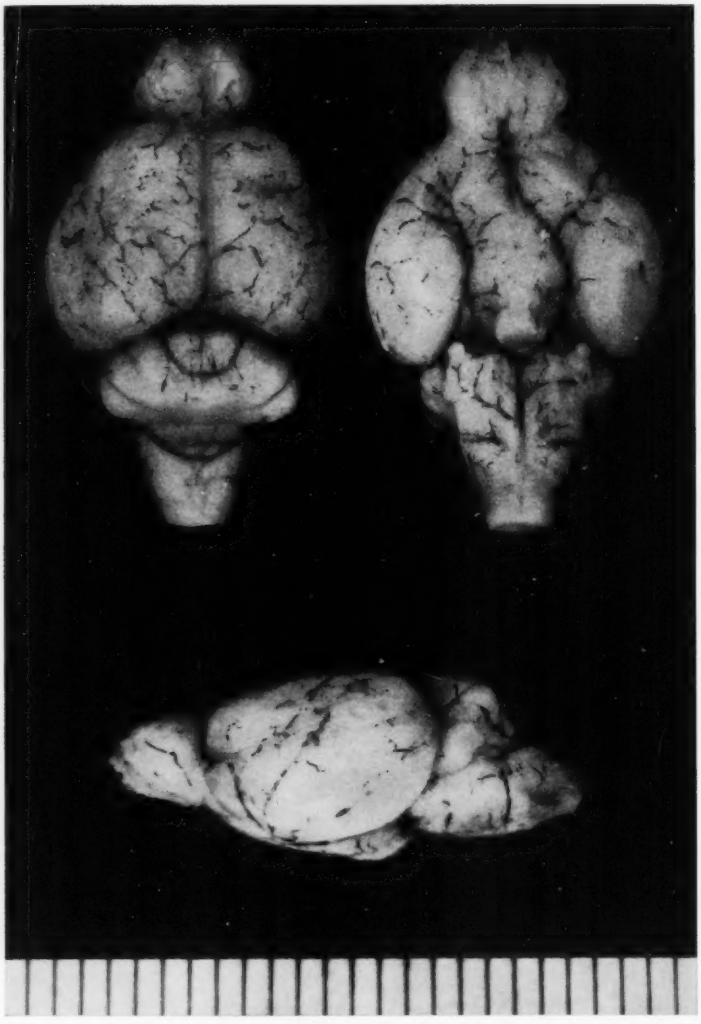

Abb. 2. Gehirn von *Heterocephalus glaber* (T 93), dorsal, basal und links-lateral aufgenommen (Masstab in mm.).

Neocortex deutlich. Die Grosshirnhemisphäre hat eine ovale Form, das Kleinhirn ist kümmerlich entwickelt und grosse Teile der Brücke und der Medulla oblongata sind frei zu sehen.

Bei *oraler Betrachtung* (Abb. 2, 3) ist die Abgrenzung der Bulbi und Tubercula olfactoria schwach ausgeprägt, der Lobus piriformis ist breit. Medial davon, im Bereich eines breiten Hypothalamus, finden sich Rudimente des Sehnerven und des optischen Chiasma, die fast intramural (eine genaue Analyse dieser

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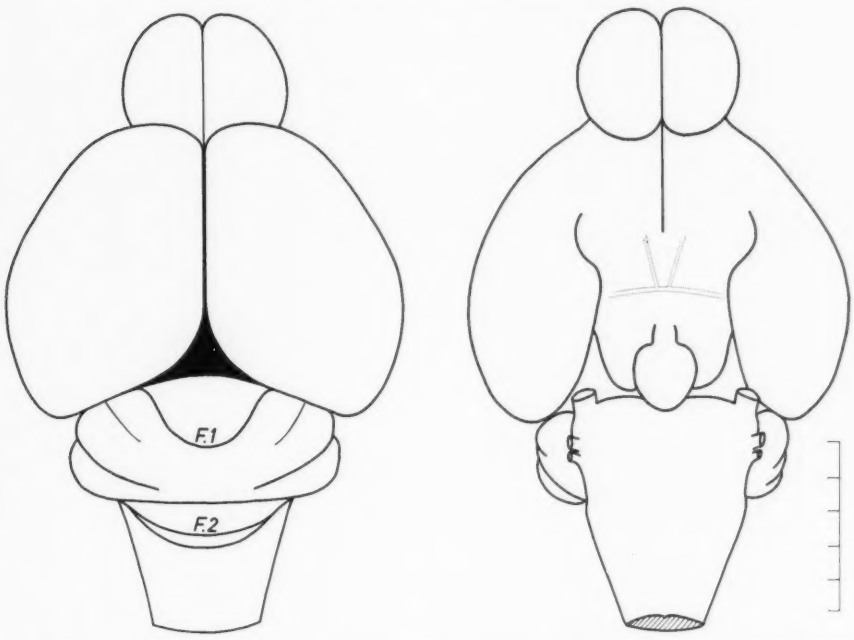
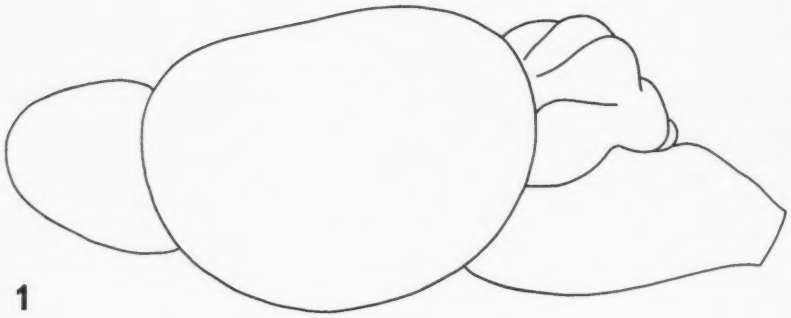


Abb. 3. Dorsale und basale Ansicht des Gehirnes von *Heterocephalus glaber* (schematisch, Masstab in mm) : *F.1* = Fissura prima, *F.2* = Fissura secunda ; Sehnervreste punktiert.

Strukturen erfolgt histologisch) als zarte, weissliche Stränge (Abb. 3, 5) verlaufen. Die Pars oralis tuberis ist lang, das Infundibulum kurz und kräftig, die Hypophyse rundlich und abgeplattet. Sie überdeckt mit ihrem caudalen Ende (Abb. 3, 5) einen schmalen Teil des freien Brückenrandes. Die Brücke geht ohne makroskopische Grenze in die Medulla oblongata über. Der Nervus trigeminus ist stark entwickelt. Das Kleinhirn überragt nicht stark die lateralen Brückenkonturen.

Kleinhirn: das Kleinhirn ist sehr primitiv gebaut, und man gewinnt den Eindruck, dass in diesem Hirnteil beim *Heterocephalus glaber* nur eine Grundstruktur vorliegt. Auf der *dorso-caudalen Fläche* (Abb. 3) ist die Fissura prima sehr deutlich, tief und grenzt einen schmalen halbkreisförmigen Lappen ab, der auf der Vierhügelplatte liegt (Abb. 4: 2). Die Crura der Kleinhirnhemisphären sind nur seitlich durch Furchen unterteilt, welche medial nicht verbunden sind. Die Fissura secunda ist sowohl seitlich als auch im medialen Bereich sehr deutlich. Cranial von dieser findet sich eine ebenso deutliche Furche — offenbar ein Homologon der Fissura praepyramidalis anderer Nagetiere. Bei *Seitenbetrachtung* (Abb. 4: *1*) ist der Lobulus flocculo-nodularis einheitlich gebaut und deutlich entwickelt. Auf dem *Mediansagittalschnitt* (Abb. 4: *2*) tritt die einfache Einteilung der Struktur deutlich in Erscheinung. Cranial der Fissura prima findet sich



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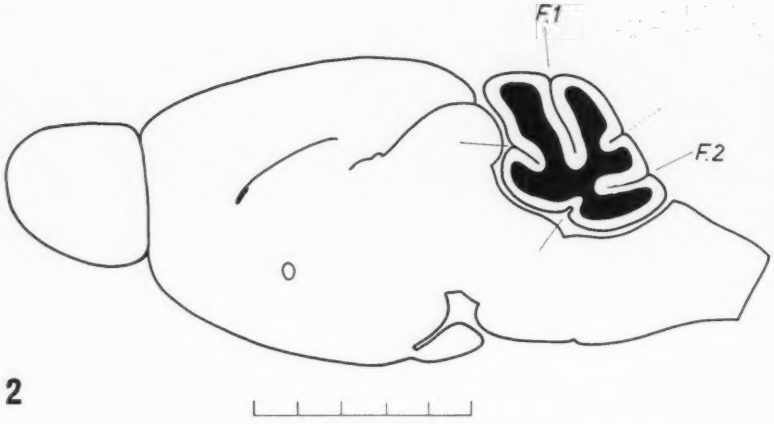

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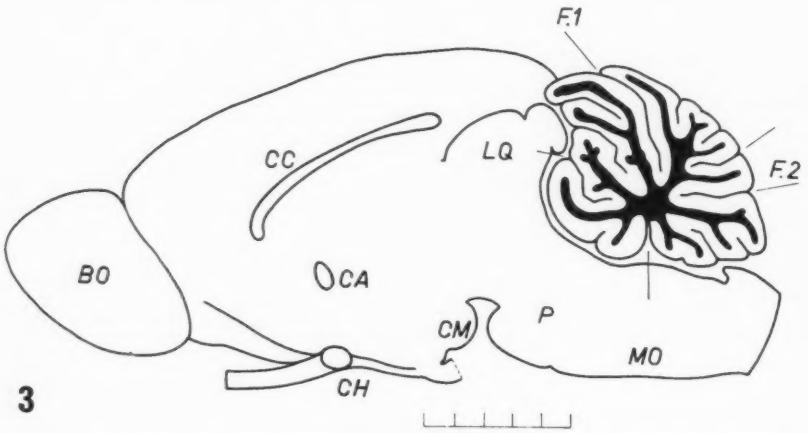

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Abb. 4. *1* und *2* = Gehirn von *Heterocephalus glaber*, laterale und mediale Ansicht. *3* = Gehirn der *Hausratte*, mediale Ansicht (Masstab in mm.) *BO* = Bulbus olfactorius, *CC* = Corpus callosum, *CA* = Commissura anterior, *CH* = Chiasma, *CM* = Corpus mammillare, *P* = Brücke, *MO* = Medulla oblongata, *LQ* = Lamina quadrigemina, *F.1* = Fissura prima, *F.2* = Fissura secunda.

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Abb. 5. Orale Fläche des Hypothalamus von *Heterocephalus glaber: NO* = Nervus opticus, *CW* = Circulus Willisi, *To* = Tractus opticus, *Pp* = Pars parainfundibularis, *Hy* = Hypophyse, *I* = Infundibulum, *Pot* = Pars oralis tuberis, *Ch* = Chiasma opticum.

noch eine tiefe Furche, die wahrscheinlich einer Fissura praecentralis entspricht. Wir wollen vorläufig von dieser einfachen Strukturierung des Cerebellums von *Heterocephalus glaber* (Vergleiche sie mit der des *Ratten*gehirnes: Abb. 4, *3*) Kenntnis nehmen und mit dem vorhandenen Material in der Homologisierung der Furchen noch vorsichtig sein. Von der *Vierhügelplatte* (Abb. 7) sind nur die Colliculi superiores gut entwickelt, die inferiores kaum ausgeprägt.

Der *Thalamus* (Abb. 7) ist gut entwickelt, dessen Längsachse schief gelegen; das *Ventrikelsystem* ist für die Dimensionen des Präparates relativ weit. Näheres über diese Strukturen wollen wir in einer späteren histologischen Arbeit untersuchen.

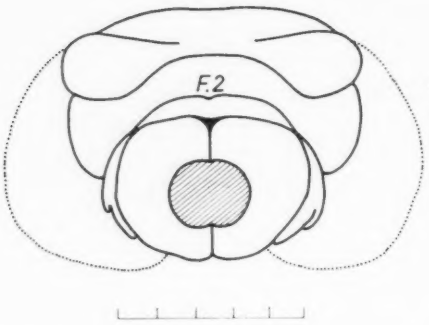

Abb. 6. Caudale Ansicht des Gehirnes von *Heterocephalus glaber*: *F.2* = Fissura secunda.

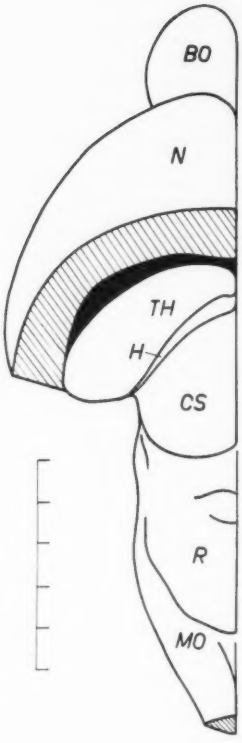

Abb. 7. Darstellung des Thalamus und der Vierhügelplatte nach Abtragung des dorsalen Neocortex und des Kleinhirns: *BO* = Bulbus olfactorius, *N* = Neocortex, *TH* = Thalamus, *H* = Habenula, *CS* = Colliculus superior laminae quadrigeminae (*Colliculus inferior* total reduziert!), *R* = Rautengrube, *MO* = Medulla oblongata.

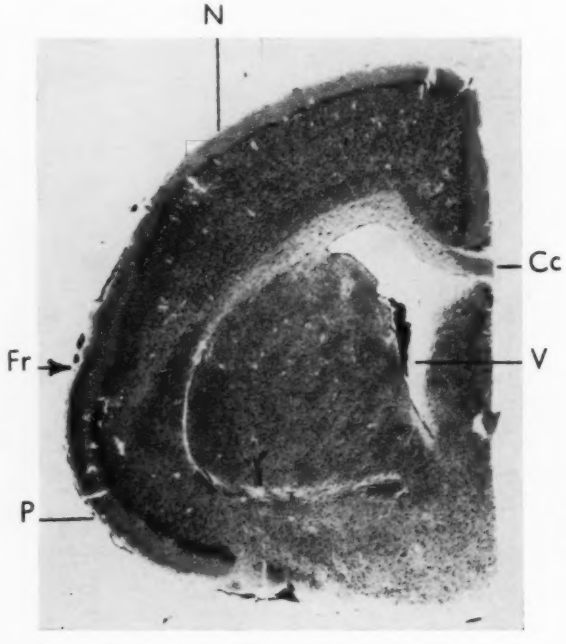

Abb. 8. Histologischer Frontalschnitt (Übersicht, Vergr. 16 ×, Färbung Kresylechtviolett): *Fr* = Fissura rhinalis, *N* = Neocortex, *P* = Palaeocortex, *Cc* = Corpus callosum, *V* = Ventrikel.

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Hirnmessungen

Grosshirnlänge	9	mm
Grosshirnbreite	11	mm
Grosshirnhöhe	7	mm
Balkenlänge	3	mm
Hypothalamuslänge	3	mm
Länge des Bulbus olfactorius	3	mm
Breite des Bulbus olfactorius	2,5	mm
Breite des Tuberculum olfactorium	3	mm
Länge des Tractus olfactorius	4	mm
Breite des Tractus olfactorius	0,3	mm
Kleinste Entfernung zwischen den Unci piriformes	3,5	mm
Hypophysenlänge	1,5	mm
Hypophysenbreite	1,5	mm
N. trigeminus-Durchmesser	1	mm
Kleinhirnbreite	7	mm
Kleinhirnlänge	4,6	mm
Kleinhirnhöhe	3	mm

$$\frac{\textit{Hypothalamuslänge}}{\textit{Grosshirnlänge}} = 0{,}33$$

Vergleichend-anatomische Betrachtungen

Es sind bisher allzuwenig Hirnformen von *Rodentia* morphologisch analysiert und deren gemeinsame Strukturzüge herausgearbeitet worden, um über die Zugehörigkeit einer neu beschriebenen Hirngestalt Bescheid zu sagen. Immerhin hat das Zentralnervensystem von *Heterocephalus glaber,* trotz extremer Rückbildung mancher Formationen (optisches System, Cerebellum), die die vergleichende Betrachtung erschwert, bei anderen Formationen strukturelle Grundzüge bewahrt, die den anatomischen Vergleich gestatten. So zum Beispiel im Hypothalamus, einem, phylogenetisch betrachtet, alten Teil des Gehirnes. Die von uns beobachteten, noch vorhandenen Rudimente des optischen Chiasma erlauben die Ausdehnung der Pars oralis tuberis zu erfassen, jenes Zwischenhirngebiet, welches zwischen Chisama und Infunibulum (Hypophysentrichter) liegt. Dieser Teil ist bei *Heterocephalus glaber,* wie bei den *Myomorpha,* lang. Bei den *Hystricomorpha* ist die Pars oralis tuberis wesentlich kürzer. Die Hypophyse überdeckt mit ihrem caudalen Ende einen Teil des freien Brückenrandes, ein Merkmal das bei *Hystricomorpha* im allgemeinen auch nicht vorkommt, weil das ganze Hypophysensystem durch die kurze Pars oralis tuberis rostraler zu liegen kommt. Der Hyptha-

lamusindex (Hypothalamuslänge: Grosshirnlänge) steht mit einem Wert von *0,33* näher den bei *Myomorpha* errechneten Quotienten als denen der *Hystricomorpha.*

Die Fissura rhinalis (palaeo-neocorticale Grenze), ein bei *Hystricomorpha* makroskopisch konstant vorkommendes Merkmal, ist bei *Heterocephalus* nur histologisch nachweisbar.

Vergleichend-hirnanatomisch betrachtet, steht *Heterocephalus* daher den *Myomorpha* näher als den *Hystricomorpha.*

Die starke Rückbildung des optischen Systems und die extrem einfache Form des Kleinhirns gewähren anderseits dem ZNS dieser Art eine Sonderstellung. An Hand dieser taxonomischen Merkmale würde *Heterocephalus glaber,* wie von Wood vorgeschlagen, einer Sondergruppe, d. h. einer weiteren Subordnung angehören.

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Zusammenfassung

Das Gehirn von *Heterocephalus glaber* (*Rodentia, Bathyergidae*) wird anatomisch untersucht: Das optische System ist noch als Rudiment vorhanden; das Kleinhirn weist nur eine sehr primitive Furchung auf; die Colliculi inferiores der Lamina quadrigemina sind extrem zurückgebildet; der Hypothalamus ist primitiv gebaut. Die Fissura rhinalis (palaeo-neocorticale Grenze) ist makroskopisch nicht sichtbar.

Vergleichend-anatomisch betrachtet weist das Gehirn von *Heterocephalus glaber* Merkmale auf, die denen der *Myomorpha* ähnlich sind. Anderseits sprechen die extremen reduzierten Hirngebiete für eine systematische Sonderstellung der Familie dieser Art, der vielleicht den Rang einer Subordnung gebührt.

Untersuchungen über weitere Vertreter der *Bathyergidae* und die histologische Analyse der Gehirne sind noch notwendig, um über diese kritische Frage der Taxonomie zu entscheiden.

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Les Batraciens anoures. Caractères ostéologiques des Discoglossidae d'Europe

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La Classe des Batraciens comporte, actuellement, deux Ordres principaux: les Urodèles (*Caudata, Gradientia*) et les Anoures (*Ecaudata, Salientia*).

La morphologie du squelette a une importance primordiale dans la Classification des Anoures, qui feront l'objet de cette étude.

Préliminaires : les Batraciens anoures actuels

Les Anoures adultes ont le corps court, dépourvu de queue, et deux paires de membres dont la paire pelvienne, la plus développée, est, le plus souvent, adaptée au saut.

I. CARACTÈRES GÉNÉRAUX DU SQUELETTE

Le crâne

Le *crâne,* court, large et aplati, est partiellement cartilagineux, même à l'âge adulte.

En avant, une pièce osseuse tubulaire entoure le crâne cartilagineux, à la naissance même des orbites, c'est le *sphénethmoïde* de W. K. PARKER ou *os en cein-*

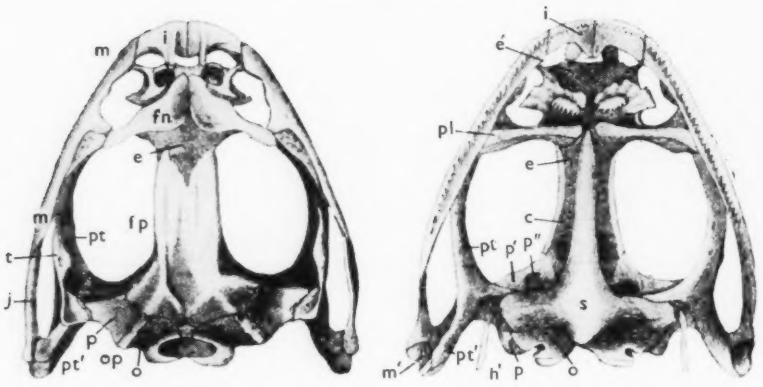

Fig. 1. Crâne de *Rana esculenta* L. (Grenouille verte) actuelle. — A gauche, vue supérieure. A droite, vue inférieure. c. mur cartilagineux du crâne. — e. sphénethmoïde (os en ceinture). — é. cartilage du nez. — f.n. nasal. — f.p. fronto-pariétal. — h' stylo-hyoïdien. — i. intermaxillaire. — j. quadrato-jugal. — m. maxillaire supérieur. — m' tractus du *quadratum*. — o. exoccipital (occipital latéral). — op. opisthotique. — p. pro-otique. — p' bras antérieur du pro-otique. — p'' foramen pour le passage du nerf trijumeau. — pl. palatin. — pt. ptérygoïde. — pt' bras postérieur du ptérygoïde. — s. parasphénoïde. — t. squamosal. 2 fois grandeur naturelle. Extrait de A. Ecker.

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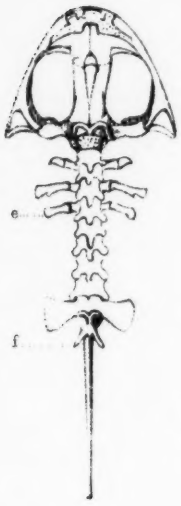
ture de G. Cuvier. Les frontaux et les pariétaux sont fusionnés en une paire de *fronto-pariétaux,* qui peuvent, eux-mêmes, se souder sur la ligne médiane (*Pelobates*). Les occipitaux sont réduits à deux *occipitaux latéraux,* qui laissent entre eux une fontanelle plus ou moins étendue et se réunissent parfois.

Le cartilage quadrato-palatin, par sa région palatine, atteint l'ethmoïde; la voûte que forme ce cartilage est recouverte par : 1) le *quadrato-jugal,* très réduit, qui unit le *quadratum* et le squamosal à l'extrémité postérieure du maxillaire ; 2) le *ptérygoïde,* robuste, qui s'étend de l'angle interne et distal du *quadratum* au maxillaire ; 3) les *palatins,* de forme et de taille variables, qui, transversaux, s'unissent sur la ligne médiane (ils manquent chez *Bombina* et *Pelodytes*).

Les *quadratums* sont dirigés transversalement et vers l'arrière, disposition en rapport avec la grande ouverture de la bouche. Le *squamosal,* développé, recouvre tout à fait le *quadratum,* du côté externe ; il présente une apophyse dirigée vers l'avant, qui s'unit à une apophyse correspondante du maxillaire pour former avec elle une arcade osseuse chez *Discoglossus,* alors que cette apophyse est à peine marquée, parfois (chez *Bufo,* par exemple). Les *os nasaux,* développés, s'unissent sur la ligne médiane. Souvent, il existe une fontanelle entre les frontaux (*Discoglossus*).

La cavité tympanique est bordée en avant, au-dessus et en dessous par le squamosal et le *quadratum* ; en arrière, par le *musculus depressor mandibulae* ; du côté interne, par la capsule otique et le cartilage du crâne.



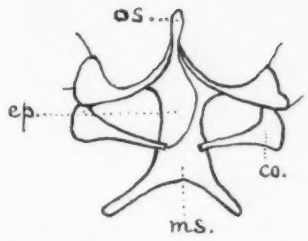


Fig. 2. (à gauche) Crâne et colonne vertébrale de *Discoglossus pictus* Otth. (Discoglosse) actuel, de Grèce. Vue dorsale. — e. côtes. — f. diapophyses du coccyx. Un peu plus grand que nature. Extrait de E. D. Cope.

Fig. 3. (à droite) Appareil sternal de *Discoglossus pictus* Otth. (Discoglosse) actuel. — co. coracoïde (partie postérieure). — ep. cartilage épicoracoïde. — ms. metasternum (xiphisternum). — os. omosternum. Schéma d'après G. A. Boulenger.

La mandibule présente, en avant, des *cartilages de Meckel* (qui manquent chez les *Discoglossidae*) ; ils demeurent cartilagineux ou sont envahis par : le *dentaire*, un petit *articulaire* et un *angulaire* interne ; il n'y a pas de splénial ; le dentaire lui-même est très réduit, alors que l'angulaire forme la majeure partie de la mandibule osseuse.

Sur les mâchoires, les dents (quand il y en a) sont disposées en une seule rangée, surtout à la mâchoire supérieure, où, souvent, intemaxillaires et maxillaires sont dentés. Les vomers peuvent aussi porter une série de dents sur leur bord postérieur.

Des ossifications dermiques se développent au niveau de la tête chez quelques espèces de *Bufo*, davantage chez les *Hemiphractinae* et, surtout, chez *Pelobates cultripes* et *Calyptocephalus*.

La colonne vertébrale

La *colonne vertébrale* de l'adulte comporte, le plus souvent, de 10 à 12 vertèbres qui, à l'exception de la première et de la dernière, présentent de fortes apophyses transverses, les diapophyses : dans leur prolongement, on rencontre, parfois, de courtes côtes (*Discoglossidae*). Les apophyses épineuses sont déprimées.

On distingue :

1°. des *vertèbres présacrées*, en petit nombre, généralement procoeles ; la 1ère, l'atlas, s'articule par deux facettes aux deux condyles occipitaux ;

2°. une *vertèbre sacrée*, rarement deux, ou sacrum ;

3°. un *coccyx* ou urostyle, allongé en forme de bâton ou de poignard, qui résulte de la fusion, au cours du développement, des vertèbres postsacrées. Il s'articule, en avant, au sacrum, par deux facettes, rarement, une seule.

Les membres

La ceinture scapulaire, bien développée en général, n'est que partiellement ossifiée. Elle n'a pas de connection réelle avec le squelette du tronc, en raison de ce que les côtes sont absentes ou rudimentaires chez l'adulte. On lui distingue : ventralement, un *coracoïde* fenestré qui s'articule au sternum ; dorsalement, un *scapulum,* qui comporte lui-même : un *suprascapulum* en partie cartilagineux, en arrière, et *l'omoplate* proprement dite, vers l'avant, qui est ossifiée et se fixe au coracoïde. Les coracoïdes sont plus ou moins unis entre eux par les « cartilages épicoracoïdes ». La pièce qui ferme la ceinture en avant est le *sternum,* lui-même divisé en deux parties : le *sternum* proprement dit (ou omosternum), qui est une tige ossifiée, et le *xiphisternum,* cartilagineux, souvent en forme de disque (Fig. 3).

La ceinture pelvienne est formée par *les ilions,* très étroits et allongés, qui s'attachent solidement aux diapophyses sacrées, et les *ischio-pubis,* unis aux ilions, fort en arrière du sacrum, qui, de chaque coté, constituent, avec ces derniers, la cavité acétabulaire destinée à la tête du fémur.

Le squelette des membres *sensu stricto* est surtout caractérisé par la fusion des os longs et celle des divers os carpiens et tarsiens.

Le *radius* et le *cubitus* sont unis en un seul os, à la patte antérieure, le *tibia* et le *péroné,* à la patte postérieure.

Au carpe, il faut surtout noter que les os de la rangée distale, 4 et 5, sont fusionnés (Fig. 4, III).

Le tarse est remarquable par l'allongement de l'astragale et du calcaneum, qui, souvent unis à leurs extrémités, forment même, parfois, un seul os : *l'astragale* semble correspondre à l'union du tibial et de l'intermédiaire, le *calcanéum,* au péronéal. Une partie des autres éléments se fusionnent entre eux : la *rangée distale* comporte encore 3 os chez les formes primitives comme *Bombina* (Fig. 4, IV), 2 seulement, chez les Anoures évolués. — L'allongement du segment tarsien proximal semble résulter d'une adaptation au saut.

Il existe moins d'os fusionnés chez les formes primitives, *Discoglossidae* et *Pelobatidae,* par exemple, que chez les Anoures spécialisés.

Les doigts

Les Anoures adultes possèdent 4 doigts, à la patte antérieure, 5, à la patte postérieure.

Il semble évident que l'extrémité antérieure des Batraciens présentait, à l'origine, 5 rayons digités. Mais, les Batraciens plus évolués que les *Embolomeri,* sous-ordre éteint qui vécut du Carbonifère au Permien (D. M. S. Watson), n'ont que 4 doigts. Toutefois, H. Steiner (1921) a signalé la présence de 5 doigts et du praepollex, à la patte antérieure de l'embryon de *Bombina.*

A l'état plus ou moins développé il existe toujours un *praepollex* et un *praehal-*

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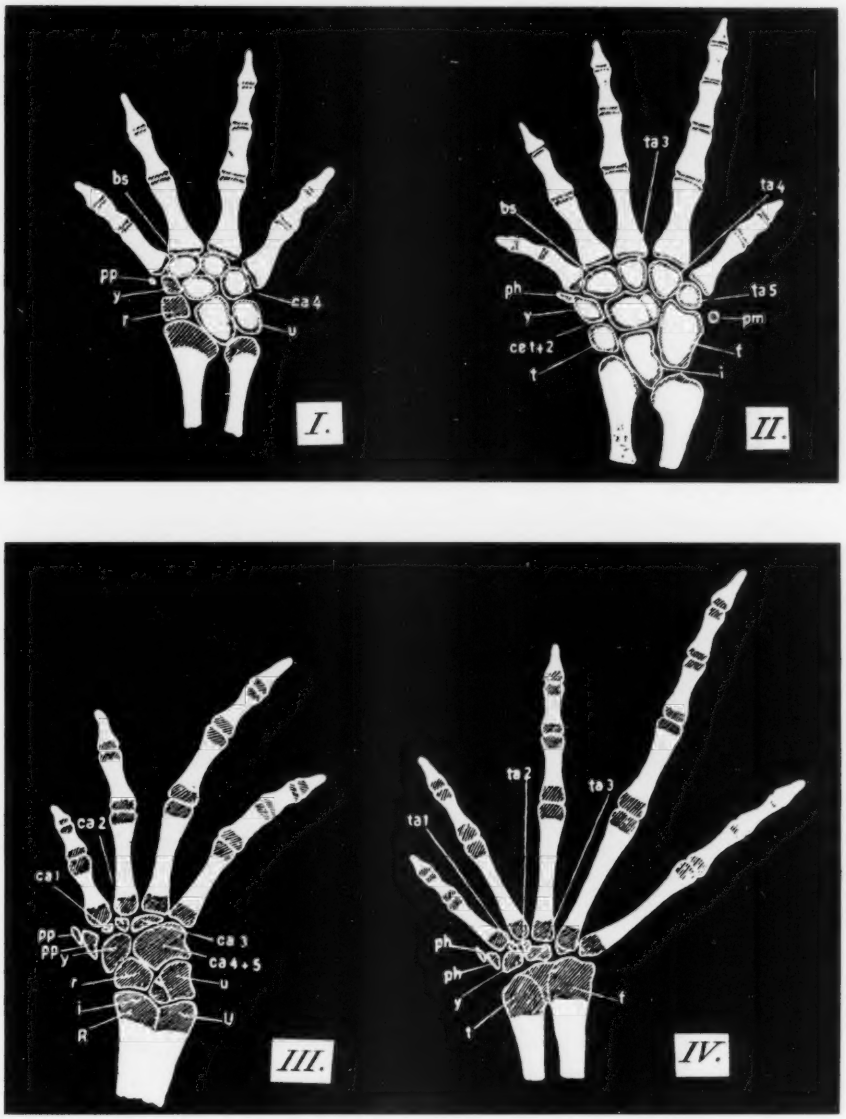

Fig. 4. Squelette d'extrémités de *BATRICIENS*, du côte droit. Les hachures indiquent le cartilage. I et II : *Ambystoma opaca* (Grav.) (Axolotl) actuelle : *URODÈLES*. I. Patte *antérieure* (longueur totale de l'Animal, du museau à l'orifice anal : 55 mm). II. Patte *postérieure* (longueur totale de l'Animal : 62 mm). 5 fois grandeur naturelle environ. III et IV : *Bombina variegata* (L.) actuelle, très jeune, avec appendice caudal : *ANOURES*. III. Patte *antérieure* (longueur totale de l'Animal : 16 mm). IV. Patte *postérieure* (longueur totale de l'Animal : 15 mm). 15 fois grandeur naturelle environ. R. radius. — U. cubitus. — r. radial. — u. cubital. — t. astragale. — f. calcaneum. — i. intermédiaire. — ce. central. — bs. *basale*. — y. *carpale praepollicis* ou *tarsale praehallucis*. — ca. carpien. — ta. tarsien. — pp. *praepollex*. — ph. *praehallux*. — pm. *postminimus*. Extrait de H. Steiner.

lux du côté interne des extrémités. Le praepollex a même été décrit chez l'embryon des Reptiles (H. STEINER, 1922).

La *formule phalangienne.*

A la *patte antérieure*, les Anoures ont, en général : 2—2—3—3 phalanges.

A la *patte postérieure* de *Discoglossus* et de *Bombina,* il existe : 2—2—3—*4*—3 phalanges (Fig. 4, IV).

Alytes, plus évolué que les deux genres précédents, possède, comme beaucoup d'Anoures : 2—2—3—*3*—3 phalanges.

II. CLASSIFICATION

Le crâne, les dents, la ceinture scapulaire et la colonne vertébrale constituent les principaux critères de classification des Batraciens anoures, bien qu'en 1922 G. K. NOBLE ait fait des objections de valeur à ces critères.

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1. Le crâne

Le degré d'ossification du crâne, qui varie avec les groupes envisagés, serait un caractère morphologique important, si le crâne était bien connu dans tous les genres d'Anoures, ce qui n'est pas, actuellement, le cas.

2. La dentition

La dentition est assez variable ; c'est ainsi que les Crapauds (*Bufonidae*) n'ont pas de dents et que les Grenouilles (*Ranidae*) n'en possèdent pas à la mâchoire inférieure. On sait que, chez les Grenouilles, les dents supérieures, formées d'ivoire recouvert d'émail, sont enkylosées à la mâchoire par l'intermédiaire d'un piédestal osseux particulier et que, du côté interne de la dent en fonction, se développe la dent de remplacement (Ch. S. TOMES). D'autre part, G. K. NOBLE a souligné que, dans certains genres, les dents inférieures ne sont que des prolongements acuminés des os mandibulaires. L'histologie dentaire des Anoures est loin d'être connue dans son ensemble et, d'après les observations de G. K. NOBLE, il est difficile, à l'heure actuelle, d'attribuer, comme l'avait fait G. A. BOULENGER, une importance générique à la dentition.

3. La ceinture scapulaire

Pendant fort longtemps, une partie des Anoures, les PHANÉROGLOSSES (qui possèdent une langue attachée au plancher de la cavité bucale), ont été divisés en F i r m i s t e r n i a (à coracoïdes fortement unis par les cartilages épicoracoïdes soudés en une seule pièce) et A r c i f e r a (à coracoïdes unis sur la ligne médiane par deux larges cartilages épicoracoïdes arqués, dont l'un recouvre généralement l'autre).

Mais, à plusieurs reprises, des auteurs ont montré qu'au cours du développement, la ceinture scapulaire des *Firmisternia* passe par un stade *Arcifera,* en sorte que les *Firmisternia* seraient les plus évolués de ces Anoures. La ceinture scapulaire de certains genres présente, d'ailleurs, une morphologie intermédiaire entre la ceinture des *Arcifera* et celle des *Firmisternia.*

4. Colonne vertébrale

G. K. Noble a insisté sur la difficulté de préparer correctement la colonne vertébrale des Anoures, afin que le caractère de chaque vertèbre soit bien respecté. Pour cet auteur, cependant, le meilleur critère ostéologique de l'Ordre entier serait certainement la colonne vertébrale.

Voici la *Classification des Batraciens anoures actuels,* proposée par G. K. Noble, en 1931, que nous adopterons.

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Ordre des Anoures

Sous-Ordre I. AMPHICOELA

Les plus archaïques. Vertèbres amphicoeles, en partie cartilagineuses, comme chez les Urodèles en général. Une seule famille :

1. LIOPELMIDAE.

Sous-Ordre II. OPISTHOCOELA

Vertèbres présacrées opisthocoeles, avec un corps fusionné au reste de la vertèbre. Côtes présentes, à quelque stade du développement.

2. DISCOGLOSSIDAE. Vertèbre sacrée libre, à corps biconvexe ; au moins 8 vertèbres présacrées. Côtes au niveau des 2ème, 3ème et 4ème vertèbres, chez l'adulte. Arcifères.

3. RHINOPHRYNIDAE (C. F. Walker 1938). Coccyx libre articulé au sacrum par deux cavités. 7 vertèbres présacrées. Pas de côtes chez l'adulte. Arcifères. Pas de sternum.

4. PIPIDAE. Vertèbre sacrée fusionnée au coccyx. De 7 à 5 vertèbres présacrées, avec côtes au début du développement. Partiellement ou totalement firmisternes.

Sous-Ordre III. ANOMOCOELA

Vertèbre sacrée procoele ankylosée au coccyx (ou libre, à un seul condyle). 8 vertèbres présacrées procoeles ou avec des disques intervertébraux libres ; sans côtes, même au cours du développement.

5. PELOBATIDAE. Crapauds fouilleurs. Diapophyses sacrées élargies. Arcifères.

Sous-Ordre IV. PROCOELA

Vertèbre sacrée libre, procoele, avec double condyle pour le coccyx. 8 à 5 vertèbres présacrées, procoeles, sans côtes.

6. BUFONIDAE. Crapauds. Arcifères. Diapophyses sacrées cylindriques ou élargies. 8 vertèbres présacrées, rarement 7. Phalanges terminales simples ou en forme de T.

7. BRACHYCEPHALIDAE. Les deux moitiés de la ceinture pectorale, partiellement ou totalement, fusionnées sur la ligne médiane. 8 à 5 vertèbres présacrées. Phalanges terminales, jamais en forme d'ongle.

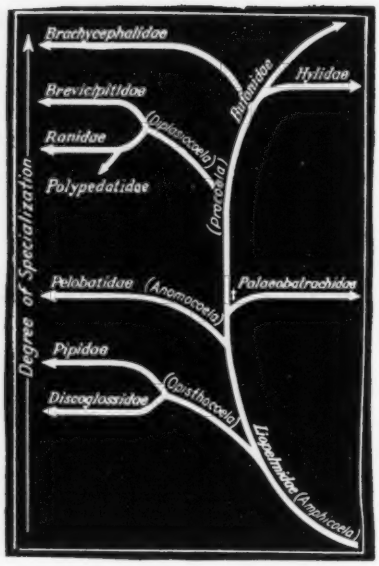

Fig. 5. Schéma de la phylogénie des BATRACIENS ANOURES. Extrait de G. K. NOBLE.

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8. HYLIDAE. Rainettes. Diapophyses sacrées généralement élargies. Cartilages intercalaires aux doigts, phalanges terminales, en forme d'ongle.

Sous-Ordre V. DISPLASIOCOELA

Vertèbre sacrée convexe antérieurement, à double condyle postérieur pour le coccyx. 8ème vertèbre biconcave, précédée de 7 vertèbres présacrées procoeles (les deux premières rarement fusionnées).

9. RANIDAE. Grenouilles. Diapophyses sacrées peu dilatées. Doigts sans cartilages intercalaires. Firmisternes.

10. POLYPEDATIDAE. Diapophyses sacrées cylindriques. Doigts avec cartilages intercalaires.

11. BREVICIPITIDAE. Les *moins spécialisés* d'entre eux, proches des *Ranidae* ; en diffèrent par leurs apophyses sacrées plus élargies. Vomers développés. — Les *plus évolués,* sans dents ; éléments ventraux de la ceinture scapulaire, réduits.

Ces préliminaires exposés, l'ostéologie des *Discoglossidae*, actuels et fossiles, parmi les *Opisthocoela,* nous intéressera seule.

Rappelons que plusieurs Anoures fossiles ont été classés « Opisthocoeles », sans rentrer dans les familles actuelles. Ce sont :

1) De l'Eocène du Geiseltal (Saxe) : l'*Opisthocoelellus,* le *Germanobatrachus* et l'*Opisthocoelorum* qui, d'après leur auteur (O. KUHN, 1941), forment une famille nouvelle, encore indéterminée ; il s'agit d'« arcifères sans côtes, à 8—7 vertèbres présacrées ».

2) Du Crétacé ou du Tertiaire inférieur d'Afrique du sud : l'*Eoxenopoïdes,*

décrit par S. H. Haughton (1931) comme le type d'une famille également indéterminée, « sans dents, à vertèbre sacréee incluse dans le coccyx ou libre ».

Il ne sera pas question, ici, de ces formes éteintes, qui ne rentrent nullement dans la famille des *Discoglossidae*.

Les Discoglossidae

C'est l'anatomie des formes actuelles qui permet d'interpréter celle des fossiles, souvent très fragmentaire. Il convient donc d'étudier, tout d'abord, les *Discoglossidae* vivants.

I. CRITÈRES OSTÉOLOGIQUES DES DISCOGLOSSIDAE ACTUELS

La Classification des Anoures souligne que les Opisthocoeles, auxquels appartiennent les *Discoglossidae*, par leurs vertèbres présacrées opisthocoeles et la présence de côtes à quelque stade du développement, sont des formes très primitives*. Ces caractères les rapprochent même des Urodèles (*Caudata*).

Chez les Têtards de *Pipidae* et de *Discoglossidae*, il existe des côtes distinctes des apophyses vertébrales. Après leur métamorphose en Adultes, les côtes se soudent aux diapophyses chez les *Pipidae*, alors qu'elles subsistent, articulées aux vertèbres, durant toute la vie, chez les *Discoglossidae*.

Les principaux caractères particuliers des *DISCOGLOSSIDAE* actuels sont les suivants.

1. Dents à la mâchoire supérieure ;
2. Colonne vertébrale
   - Au moins 8 *vertèbres présacrées* ;
   - *Vertèbre sacrée* libre, à corps biconvexe, à diapophyses plus ou moins élargies ;
   - *Coccyx* articulé au sacrum par deux cavités cotyloïdes (une seule chez *Bombina*) ; deux diapophyses divergeant vers l'arrière, à sa base ;
3. Courtes côtes articulées aux diapophyses des 2ème, 3ème et 4ème vertèbres, chez l'adulte ;
4. Xiphisternum à deux styles grêles, fibro-cartilagineux ou cartilagineux, divergeant vers l'arrière ;
5. Ceinture scapulaire arcifère ;
6. Phalanges terminales des doigts, simples, non en forme de griffe.

* Seuls, les Amphicoeles sont, actuellement, considérés comme plus archaïques.

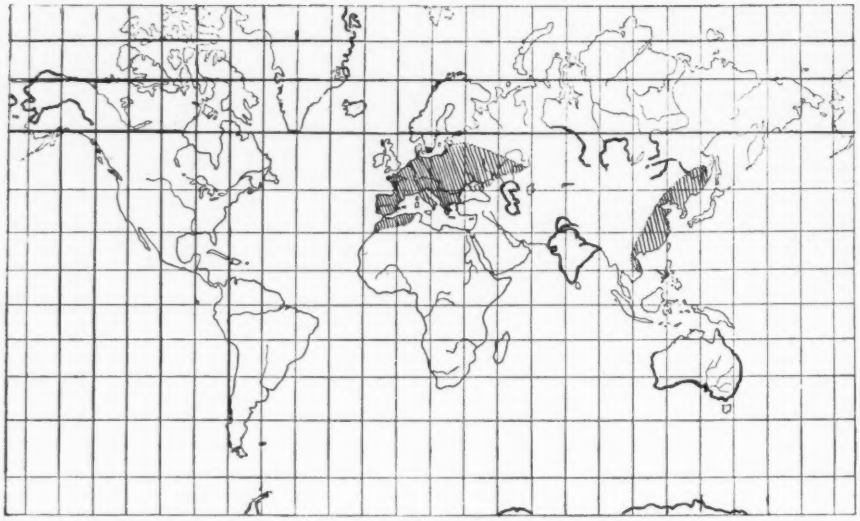

Fig. 6. Répartition géographique (en hachures) des *Discoglossidae* actuels.

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II. REPARTITION DES DISCOGLOSSIDAE DANS LE TEMPS ET DANS L'ESPACE

1. Répartition géographique des Discoglossidae actuels

Les *Discoglossidae*, aujourd'hui répandus en Europe, dans le nord-ouest de l'Afrique, en Asie orientale et dans une des îles Philippines, comportent quatre genres : *Discoglossus*, *Bombina*, *Alytes* et *Barbourula*.

Discoglossus est représenté par une seule espèce, *Discoglossus pictus* Otth., répandue du sud de la France jusqu'en Espagne, en Sicile, quelques îles de la Méditerranée et le nord-ouest de l'Afrique.

Bombina compte deux espèces en Europe : *Bombina bombina* (L.) (= *ignea* (L.)), qui s'étend du sud de la Suède et du 57ème degré de latitude nord, en Russie, jusqu'en Italie et dans les Balkans ; vers l'est, jusqu'à l'Oural ; *Bombina variegata* (L.) (= *pachypa* (Bon.)), répandue, de la France jusqu'en Bohême, dans les Karpathes et en Italie du nord. — Deux autres espèces habitent le nord-est de l'Asie, de la Mandchourie au sud-est de la Chine et au Tonkin.

Alytes, genre uniquement européen, comporte deux espèces : *Alytes obstetricans* Laur., répandue dans l'ouest de l'Europe, et *Alytes Cisternasii* Bosca, localisée à la Péninsule ibérique.

Barbourula, connue de l'île Busuanga, dans les Philippines, est représentée par une seule espèce.

2. *Répartition géographique et géologique des Discoglossidae d'Europe*

Les *Discoglossidae* fossiles n'ont été trouvés qu'en Europe. Certains se rapportent à des genres encore actuels, d'autres, à des genres éteints.

Voici un schéma de leur répartition, avec la liste des formes encore vivantes.

TERTIAIRE	QUATERNAIRE (Actuel)		S. Europe, N. O. Afrique	*Discoglossus pictus* Otth.
			Europe	*Bombina bombina* (L.).
			Europe	*Bombina variegata* (L.)
			Europe	*Alytes obstetricans* Laur.
			Pén. ibérique	*Alytes Cisternasii* Bosca
	Pliocène	supérieur / inférieur	Bassin de Vienne	*Alytes Troscheli* (Meyer)
	Miocène	supérieur		
		moyen	Œningen, Bade	*Pelophilus Agassizi* Tschudi
				Latonia Seyfriedi Meyer
			Sansan, Gers, France	*Latonia gigantea* Lartet
		inférieur	Rott, Prusse rhén.	*Alytes Troscheli* (Meyer)
				Zaphrissa eurypelis Cope
			Bassin de Mayence	*Discoglossus*
	Oligocène	supérieur	Limagne, France	*Prodiscoglossus Vertaizoni* Friant
		inférieur		
	Eocène			

Les indications données par plusieurs auteurs au sujet des genres actuels trouvés à l'état fossile seraient, sans doute, à réviser. C'est ainsi que la figure de H. v. Meyer (1860) concernant l'*Alytes Troscheli* permet difficilement d'établir les caractères de l'espèce fossile.

III. PRINCIPAUX CARACTERES OSTEOLOGIQUES DIFFERENTIELS DES DISCOGLOSSIDAE ACTUELS D'EUROPE

D'après G. A. Boulenger

	DISCOGLOSSUS	*BOMBINA*	*ALYTES*
1. Dents vomériennes	série rectiligne, en arrière des choannes	série interrompue en arrière des choannes	série ininterrompue en arrière des choannes
2. Fontanelle fronto-pariétale	petite	développée	développée
3. Préfrontaux	largement au contact	au contact antérieurement	tout à fait au contact
4. Omosternum	rudimentaire	absent	absent
5. Sacrum	2 condyles pour le coccyx	1 condyle pour le coccyx	2 condyles pour le coccyx
6. Diapophyses sacrées	peu élargies	fortement élargies	assez élargies

7. Orteils	—	extrémité non dilatée	orteils courts
8. Quand le membre postérieur est porté vers l'avant, le long du corps, l'articulation tibio-tarsienne arrive	entre l'œil et l'extrémité du museau	entre l'épaule et l'œil	au niveau du tympan

IV. LES DISCOGLOSSIDAE DU TERTIAIRE D'EUROPE

A moins d'indication contraire, nous étudirons les formes fossiles par ordre stratigraphique.

1. Prodiscoglossus

Prodiscoglossus Vertaizoni Friant

Décrit par l'auteur en 1944 (C. R. Acad. Sci. *219* : 561—562), le *Prodiscoglossus Vertaizoni* vécut au Stampien (Oligocène supérieur), dans la Limagne méridionale, entre l'Allier et la Dore, à Vertaizon (Puy-de-Dôme). Ses restes ont été trouvés dans des couches calcaires, bien connues au point de vue géologique (J. Giraud, 1902).

Le fragment-type appartient à la Faculté des Sciences de Clermont-Ferrand.

Fragment-type

C'est un échantillon de roche calcaire comportant deux squelettes, dont l'un, en très bon état, pour un squelette de Batracien, a été décrit par l'auteur en 1944. Il s'agit d'une pièce de la Collection Constancias (1896), remise à la Faculté des Sciences de Clermont-Ferrand avec le legs Duchassaint.

Horizon

Calcaire stampien (oligocène supérieur) de Vertaizon (Puy-de-Dôme).

Diagnose

Ordre des Batraciens anoures (*salientia*) par la morphologie du coccyx.
Sous-Ordre des *Opisthocoela* par :
les vertèbres présacrées opisthocoeles ;
la présence de côtes chez l'adulte.
Famille des *Discoglossidae* par :
la vertèbre sacrée non fusionnée au coccyx ;
les 8 vertèbres présacrées ;
les dents de la mâchoire supérieure.

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Fig. 7. *Prodiscoglossus Vertaizoni* Friant. Type (à droite) et co-type (à gauche) inclus dans un fragment de roche calcaire, d'âge stampien (oligocène supérieur), du gisement de Vertaizon (Puy-de-Dôme). Collection Constancias 1896, du Laboratoire de Géologie, Université de Clermont-Ferrand. Moitié de la grandeur naturelle environ. Photographie A. Breuilly. Clermont-Ferrand (Puy-de-Dôme).

Description de l'exemplaire-type

L'exemplaire-type, en bon état de conservation, vu par sa face ventrale, est de grande taille : 10 centimètres environ, de la partie antérieure du crâne, au bord postérieur des ischio-pubis. La tête est à peu près le tiers de la longueur totale : son contour est bien marqué. Beaucoup d'ossements sont distincts ; cependant, la ceinture scapulaire est fragmentaire et : du coccyx, des os de la ceinture pelvienne et du fémur droit, il ne subsiste guère que des empreintes.

Colonne vertébrale. L'*atlas* est incomplet ; les *autres vertèbres présacrées,* opisthocoeles, ont des apophyses nettes ; celles de la dernière présacrée, la 8ème, sont dirigées vers l'avant. Les diapophyses des 2ème, 3ème et 4ème vertèbres présentent de courtes *côtes,* dont la paire la plus allongée semble être la dernière, la 3ème, comme chez *Alytes* (alors que c'est la 2ème paire dans les autres genres actuels d'Europe). Le *sacrum,* constitué d'une seule vertèbre, possède des diapophyses non élargies. Le *coccyx,* de forme typique, en bâton, dont l'extrémité postérieure n'est pas nette, à deux cavités articulaires pour le sacrum, semble présenter, du côté droit, la trace d'une diapophyse divergente, à sa base.

Dents : visibles sur le bord de la mâchoire supérieure, elles sont nombreuses, petites et aigües.

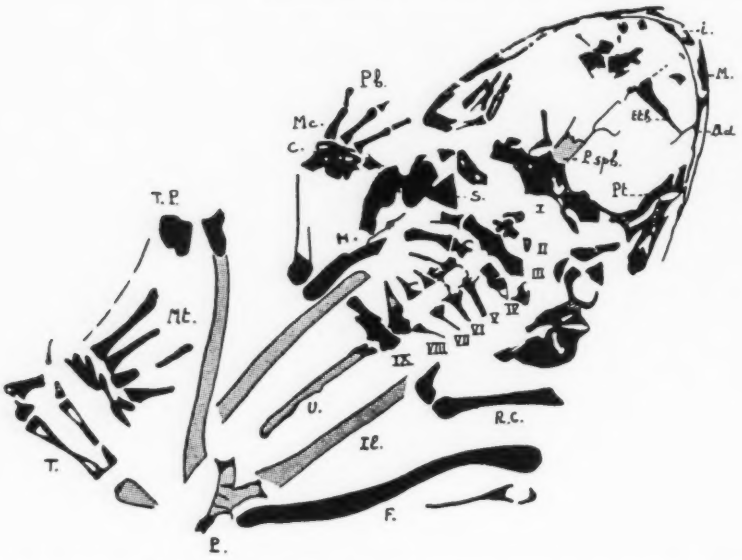

Fig. 8. *Prodiscoglossus Vertaizoni* Friant. Type, du calcaire stampien de Vertaizon (Puy-de-Dôme). Schéma de la figure 7. Les os sont en noir, les empreintes, en grisé. Ces restes semblent appartenir à un même sujet vu par sa face ventrale. c. carpe. — Eth. ethmoïde. — F. fémur. — H. humérus. — i. intermaxillaire. — Il. ilion. — M. maxillaire supérieur. — Mc. métacarpe. — Md. mandibule. — Mt. métatarse. — P. ischio-pubis. — Ph. phalange basale. — Psph. parasphénoïde. — Pt. ptérygoïde. — R. C. radio-cubitus. — S. ceinture scapulaire (coracoïde *pro parte*). — T. tarse proximal. — T.P. tibio-péroné. — U. coccyx (urostyle). — I, II, III, IV, V, VI, VII, VIII, vertèbres présacrées. — IX, vertèbre sacrée. Grandeur naturelle environ. Extrait de M. Friant. C. R. Acad. Sci. Paris, *219* (1944).

Membres. La trace des *ilions* et des ischio-pubis est marquée. Des deux côtés, le *fémur* est très allongé pour un fémur de Discoglossidé. — A la *patte postérieure* droite, l'*astragale* et le *calcaneum,* déplacés par rapport ou reste de l'extremité, sont allongés et unis dans leur région proximale. Les os tarsiens de la rangée distale et le préhallux sont marqués, les 4 premiers métatarsiens et la trace du 5ème, distincts. Les orteils ne sont pas conservés, mais il existe l'indication de la phalange basale du 5ème.

Il subsiste une partie du *coracoïde* droit. Le *radio-cubitus* gauche est court. A la *patte antérieure* droite, quelques os *carpiens* et le prepollex sont nets, les 4 *métacarpiens* (1, 2, 3, 4), conservés. Le pollex n'est pas visible ; le 2ème doigt est représenté par sa phalange basale. Au niveau du 3ème rayon, une phalange terminale, déplacée, se trouve en continuité avec le métacarpien ; le déplacement de cette phalange est confirmé par la présence des 2 premières phalanges du 3ème doigt, chez l'exemplaire co-type. Le 4ème doigt est représenté par sa phalange basale.

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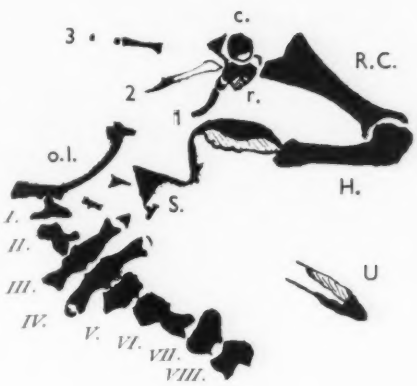

Fig. 9. *Prodiscoglossus Vertaizoni* Friant. Co-type, du calcaire stampien de Vertaizon (Puy-de-Dôme). Schéma de la figure 7. Sujet encore jeune, vu par sa face ventrale. c. os cubital. — H. humérus. — o.l. occipital latéral. — r. os radial. — R.C. radio-cubitus. — S. ceinture scapulaire (coracoïde *pro parte*). — U. coccyx (urostyle). — I, II, III, IV, V, VI, VII, VIII, vertèbres présacrées, IX, vertèbre sacrée : corps ; au niveau des vertèbres III et IV : diapophyses et indication proximale des côtes. — 1, 2, 3, métapodes antérieurs, du côté interne. Une fois et demie grandeur naturelle environ.
Nota bene. Les vertèbres VI et VII ne sont pas distinctes l'une de l'autre. Le chiffre IX de la dernière vertèbre (vertèbre tacrée) a été ornis.

Description de l'exemplaire co-type

Sur le même fragment de roche que l'exemplaire-type (Fig. 7), existent les restes d'un autre *Prodiscoglossus Vertaizoni,* de taille un peu plus faible que le type : il s'agit d'un sujet encore jeune, vu également par sa face ventrale. Cet exemplaire es décrit, ici, pour la première fois (Fig. 9).

Crâne. Il ne subsiste que les *occipitaux latéraux,* fragmentaires, avec les deux condyles occipitaux.

Colonne vertébrale. Elle est représentée par le corps des 9 vertèbres : 8 présacrées, opisthocoeles, et 1 sacrée ; la 3ème et la 4ème vertèbres possèdent, en outre, leurs diapophyses, incomplètement ossifiées, avec la trace proximale des *côtes.* L'extrémité postérieure du *coccyx,* nettement acuminée, est fracturée et déplacée.

Membre antérieur. La *ceinture scapulaire,* dissociée, est déplacée. Du côté gauche, l'*humérus* et le *radio-cubitus,* courts, sont articulés l'un à l'autre. — A la *patte antérieure,* du même côté, quelques os *carpiens* et le prépollex sont nets, les 2 *métacarpiens* internes, également. Des doigts, il subsiste : la lère phalange du 2ème doigt, l'indication des 2 phalanges basales du 3ème doigt.

Voici quelques dimensions, prises sur la photographie grandeur naturelle de l'exemplaire-type et, à titre de comparaison, celles de l'exemplaire co-type, sujet jeune, nettement plus petit que le type.

Discoglossus Vertaizoni FRIANT

	Type	Co-type
Longueur du crâne	32 mm	
Largeur du crâne	41 mm	
Longueur vertébrale totale (9 vert.) (sans le coccyx)	30 mm	25 mm
Largeur de la 8ème vertèbre avec ses diapophyses	12 mm	
Longueur du coccyx	20 mm	
Longueur de la 3ème côte	6 mm	
Longueur de l'ilion	31 mm	
Longueur du fémur	44 mm	
Longueur de l'astragale et du calcaneum	15 mm	
Longueur du 1er métatarsien	12 mm	
Longueur du l'humérus	a	14 mm
Longueur du radio-cubitus	15 mm	12 mm
Longueur du 2ème métacarpien	5 mm	4 mm

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Rapports morphologiques du Prodiscoglossus Vertaizoni avec les Discoglossidae actuels

De même que chez *Alytes,* le crâne de *Prodiscoglossus* atteint, environ, le tiers de sa longeur totale : il est, relativement, plus petit dans les autres genres d'Europe.

Les 8 vertébres présacrées du fossile et son sacrum, à double condyle pour le coccyx, le rapprochent des autres *Discoglossidae,* à l'exception de *Bombina,* qu'il rapelle, cependant, par les diapophyses de sa dernière vertèbre présacrée, dirigées vers l'avant, comme chez un grand nombre d'Anoures.

Le *Prodiscoglossus Vertaizoni* synthétise, en somme, beaucoup de caractères des *Discoglossidae* actuels d'Europe. Il en diffère, cependant, par :

1) sa grande taille : 100 mm environ, alors que le *Discoglossus,* le plus grand des genres actuels de nos régions, atteint 60 à 75 mm seulement ;

2) la longueur des membres postérieurs ;

3) ses diapophyses sacrées non élargies.

Rappelons que, pour H. W. PARKER (1931), l'*élargissement des diapophyses sacrées* aurait une signification fonctionnelle. Ces diapophyses reçoivent la poussée des membres postérieurs et la transmettent à l'axe du squelette, au cours de la progression. Il semble logique de penser que les différents types de locomotion se réfléchissent sur leur forme.

Chez des Grenouilles, qui progressent par sauts, l'impulsion des membres postérieurs varie avec les bonds, élevés ou plus réduits. Afin de résister à ces tensions diverses, la meilleure structure est incontestablement un cylindre ; il est remarquable que les apophyses sacrées des « Grenouilles des prés » soient, en effet, plus ou moins cylindriques.

D'autre part, chez les Anoures qui nagent, qui fouissent, qui grimpent, la plus grande résistance aux mouvements postérieurs doit être dans une direction parallèle à l'axe de la colonne vertébrale. Or, les diapophyses sacrées de ces Batraciens sont élargies dans la direction de cet axe, en sorte qu'il existe une surface plus développée pour une attache solide des ilions.

De ceci, il semble résulter que le *Prodiscoglossus* bondissait dans les prairies de la Limagne, alors que les *Discoglossidae* actuels sont, surtout, aquatiques. — Ces conclusions sont renforcées par la présence de fémurs très allongés chez le fossile, qui marquent aussi une adaption au saut indiscutable.

Parmi les *Discoglossidae* fossiles plus récents que le *Prodiscoglossus,* il faut distinguer :

1. Ceux qui semblent identiques aux actuels ou très proches d'eux :

Discoglossus sp.

Alytes Troscheli (Meyer).

Il est difficile de les séparer des formes vivantes, en raison de la description sommaire des auteurs.

2. Une forme éteinte : *Pelophilus,* qui se place assez nettement dans la lignée : *Prodiscoglossus* — *Discoglossidae* actuels.

3. Un groupe homogène et abezzant de formes éteintes : *Latonia* et *Zaphrissa.*

2. *Pelophilus*

Pelophilus Agassizi Tschudi

Signalé par L. Agassiz, en 1835, comme : *Bombinator Œningensis* Ag. (Mém. Soc. Sci. nat. Neuchâtel, p. 27), il fut décrit sous le nom de *Pelophilus Agassizi* par J. J. von Tschudi, en 1839 (Classification der Batrachier. Mém. Soc. Sci. nat. Neuchâtel. T. 2 : 22, 47, 84).

En 1845, H. von Meyer l'a étudié avec quelques détails. (Zur Fauna der Vorwelt).

Fragment-type

De la molasse d'eau douce d'Œningen (Bade), près du lac de Constance.

Horizon

Miocène moyen.

Description de l'exemplaire-type

Pelophilus Agassizi Tschudi, un peu plus petit que *Bombina,* s'en rapproche ; il se rapproche aussi d'*Alytes.*

Crâne. Il faut surtout noter que les pariétaux, développés en arrière, au niveau de leur union avec les occipitaux, sont étroits en avant, où ils forment un triangle allongé.

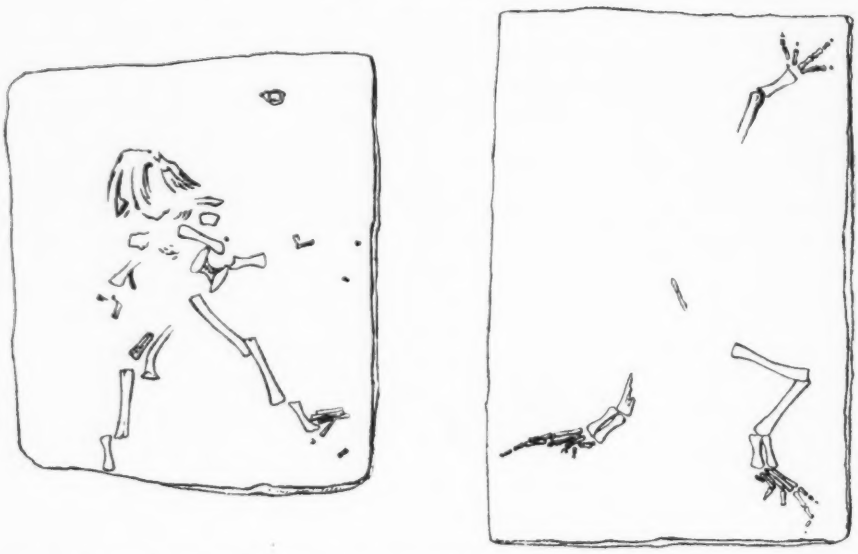

Fig. 10. *Pelophilus Agassizi* Tschudi, de la marne d'eau douce d'Œningen (Bade). Miocène moyen. Deux exemplaires très incomplets. Grandeur naturelle environ. Extrait de H. von Meyer.

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Colonne vertébrale. Les diapophyses du sacrum, élargies, ressemblent à celles de *Bombina*.

Membres. Le *fémur* est très allongé par rapport aux autres os du membre postérieur. Les extrémités sont plus fortes et plus courtes que celles de *Bombina* : l'*astragale* et le *calcaneum*, surtout, sont très courts.

Voici quelques mensurations données par H. von Meyer :

Longueur de l'humérus	8 mm
Largeur inférieure de l'humérus	2 mm
Longueur du radio-cubitus	6 mm
Longueur de l'ilion	9 mm

Autres exemplaires connus

Collection Agassiz, Neuchâtel, Suisse.

Cabinet d'Histoire naturelle, Karlsruhe, Allemagne.

3. Latonia

A. Latonia Seyfriedi Meyer

Signalée par H. von Meyer, en 1843 (*Latonia Seyfriedi* und *Pelophilus Agassizi* von Œningen. Neues Jahrb. Min. Geol. Pal. : 580) ; puis décrite et figurée

par l'auteur, en 1845 (Fauna der Vorwelt. Fossile Säugethiere. Vögel und Reptilien aus dem Molasse-Mergel von Œningen. Frankfurt-a-M. : p. 18, Pl. IV).

Fragment-type

De la molasse d'eau douce d'Œningen (Bade), près du lac de Constance.

Horizon

Miocène moyen.

Description de l'exemplaire-type

De grande taille : 170 mm environ, de l'extrémité antérieure du crâne, au bord postérieur du bassin.

Crâne. Il est court, arrondi vers l'avant, avec des fronto-pariétaux complètement ossifiés. Les orbites, petites, sont situées vers le milieu du crâne. Il existe des *ossifications dermiques* et les fosses temporales sont recouvertes par une lame osseuse. Les *dents* de la mâchoire supérieure, très caractéristiques, sont allongées, aplaties et serrées les unes contre les autres.

Colonne vertébrale. Elle comporte 8 *vertèbres présacrées*. Le *sacrum*, à deux condyles articulaires pour le coccyx, présente des diapophyses peu élargies sur l'exemplaire-type, plus larges sur les exemplaires du Polytechnicum fédéral de Zürich et du Musée de Constance. Il existe des *côtes* au niveau des 2ème, 3ème et 4ème vertèbres, avec de courts processus dirigés vers l'arrière.

Les vertèbres présacrées furent décrites comme procoeles par H. von Meyer, en 1845, mais E. D. Cope, en 1865, puis en 1867, montra que ce fossile pouvait être rapporté aux *Discoglossidae* par la présence de diapophyses à la base du coccys, visibles sur la figure-type de H. von Meyer. Sur cette figure, la 2ème vertèbre en avant du sacrum est nettement opisthocoele.

Membres. A leur niveau, l'*ilion*, légèrement courbé vers le plan médian, sur l'exemplaire-type, est plus nettement incurvé, du côté gauche, sur l'exemplaire du Polytechnicum fédéral de Zürich, par exemple. Cet os est sensiblement aussi long que le fémur.

Autres exemplaires connus

Collection du Musée zoologique de l'Université de Zürich (exemplaire sans crâne, figuré par H. von Meyer).

Collection du Polytechnicum fédéral de Zürich.

Musée Rosengarten, à Constance, Bade.

Collection du British Museum natural History.

Voici quelques dimensions se rapportant à l'exemplaire du Polytechnicum fédéral de Zürich.

Fig. 11. *Latonia Seyfriedi* Meyer, d'Œningen (Bade). Miocène moyen. Exemplaire du Polytechnicum fédéral de Zürich. Photographie du Polytechnicum.

Latonia Seyfriedi Meyer, d'Œningen (Bade)

	Longueur	Largeur
Longueur totale : de l'extrémité antérieure du crâne à l'extrémité postérieure du bassin . . .	170 mm	
Longueur du crâne	55 mm	
Largeur du crâne		80 mm
Largeur des dernières vertèbres présacrées avec leurs diapophyses		30 mm
Largeur de la vertèbre sacrée, avec ses diapophyses		30 mm
Longueur du coccyx	52 mm	
Longueur de l'ilion	54 mm	
Longueur du fémur	55 mm	
Longueur du tibio-péroné	60 mm	
Longueur de l'astragale et du calcaneum	32 mm	
Longueur de l'humérus	34 mm	
Longueur du radio-cubitus	25 mm	

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Ces mensurations sont très proches de celles indiquées par H. von Meyer pour l'exemplaire-type.

B. Latonia gigantea (Lartet)

Sommairement décrite par E. Lartet, en 1851 (Notice sur la colline de Sansan. J. A. Portes, Auch, p. 41).

Synonymie

Rana gigantea Lartet, loc. cit. 1851.

Rana rugosa Lartet, loc. cit. 1851. — P. Gervais. Zoologie et Paléontologie françaises, Paris, 1848—52, p. 494.

Latonia rugosa Cope. Sketch of the primary groups of Batrachia Salientia. Nat. hist. Rev. n° 1 (1865).

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Fragment-type

De la colline de Sansan (Gers), France.

Horizon

Miocène moyen.

Exemplaire-type

Très brièvement décrit par E. Lartet, provisoirement séparé de *Latonia Seyfriedi* Meyer.

Anoure volumineux, de 32 à 40 centimètres de longueur totale. Les mâchoires supérieures sont dentées « en peigne » et hérissées extérieurement d'aspérités, qui leur donnent l'aspect celluleux. Les os de la face sont légèrement rugueux et les fosses temporales, recouvertes par une lame osseuse. D'après E. D. Cope (1865), les vertèbres présacrées sont opisthocoeles.

Autre exemplaire connu

Collection du British Museum natural History.

4. Zaphrissa

Zaphrissa eurypelis Cope

Décrite par E. D. Cope, en 1867 (On the structure and distribution of the Genera of Arciferous Anura. Journ. Acad. nat. Sci. Philad. ser. 2, *6:* 77—79). Bel exemplaire de Discoglossidé, proche de *Latonia,* d'après l'auteur.

Fragment-type

De la lignite de Rott (Prusse rhénane).

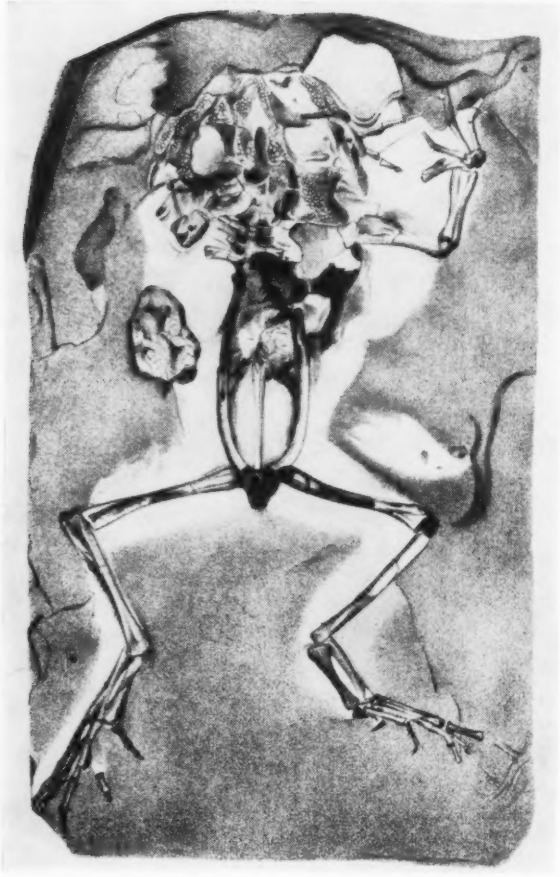

Fig. 12. *Zaphrissa eurypelis* Cope, de la lignite de Rott (Prusse rhénane). Miocène inférieur. Extrait de E. D. Cope.

Horizon

Miocène inférieur.

Description de l'exemplaire-type

L'aspect général du squelette est bien visible sur la photographie de E. D. Cope (Fig. 12).

Le crâne est large, avec une surface rugueuse, par suite du développement, à l'intérieur du derme, de petites crêtes calcifiées, nombreuses, formant un dessin noduleux, plus large que dans les formes récentes où elles existent. Les fosses temporales sont recouvertes d'une lame osseuse. Il semble y avoir une fontanelle fronto-pariétale.

Au niveau de la colonne vertébrale, l'atlas est distinct, élargi antérieurement pour l'articulation avec les deux condyles occipitaux, qui sont volumineux.

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— Les *diapophyses des 2ème, 3ème et 4ème vertèbres* sont, seules, conservés. Celles de la *2ème,* les plus longues, s'articulent, sans présenter de constriction, avec les côtes, cylindriques, dont la portion terminale a disparu. La *3ème* paire de diapophyses, la plus courte, est légèrement déprimée au niveau de l'articulation avec les *côtes,* qui sont *les plus longues,* presque droites, un peu élargies distalement. Les *4èmes* diapophyses égalent les 3èmes mais sont plus épaisses et dirigées vers l'avant ; leurs côtes, transverses, sont plus courtes que les précédentes. — A partir de la 5ème vertèbre, la colonne est déplacée. — Les *apophyses sacrées,* élargies, s'articulent avec les ilions. — Le sacrum s'unit au coccyx par deux condyles ; l'empreinte du *coccyx,* qui ne présente pas de forte carène dorsale, est bien dans l'axe du sacrum.

Le membre antérieur est, relativement, allongé. — Le *scapulum* et le suprascapulum sont indivis. — L'*humérus,* large dans sa région proximale, est comprimé, distalement, dans le sens transverse. — Le *radio-cubitus* présente deux sillons distaux longitudinaux. — Les empreintes des *os carpiens* sont nettes ; les doigts, allongés.

Le sternum est détérioré.

Au niveau du membre postérieur : les *ilions,* légèrement incurvés vers la plan médian, comprimés distalement, sont plus longs que les *fémurs.* Le *tibio-péroné,* relativement mince, aplati, plus long que l'*astragale* et le *calcaneum,* est sillonné à ses deux extrémités. — Des *métatarsiens* longs (ou externes), juxtaposés, l'externe est le plus court des deux. — Les *phalanges* terminales des orteils sont courtes et coniques.

Voici quelques dimensions de l'exemplaire-type, d'après E. D. Cope.

Zaphrissa eurypelis Cope, de Rott (Prusse rhénane)

Longueur, de l'extrémité antérieure de la tête aux condyles occipitaux	27 mm 5
Largeur de la 3ème vertèbre, avec ses diapophyses	17 mm 6
Largeur de la 4ème vertèbre, avec ses diapophyses	17 mm 6
Largeur de la vertèbre sacrée, avec ses diapophyses	12 mm 7
Longueur du coccyx (qui semble incomplet)	19 mm 8
Longuer de l'ilion	34 mm 9
Longueur du fémur	31 mm
Longueur du tibio-péroné	31 mm
Longueur de l'humérus	22 mm
Longueur du radio-cubitus	16 mm
Longueur du 3ème métacarpien	7 mm

Considérations générales

La taille relativement faible de cet exemplaire et quelques détails anatomiques : la présence d'une fontanelle fronto-pariétale, l'absence de diapophyses coccy-

giennes (encore cartilagineuses, sans doute), permettent de penser qu'il s'agit d'un sujet jeune, très probablement de *Latonia*. Les proportions des membres postérieurs, les sillons longitudinaux des os longs, rapellent également l'Anoure géant d'Œningen. Toutefois, nous conservons à cet Animal, provisoirement, au moins, le nom de *Zaphrissa eurypelis* donné par E. D. Cope.

Conclusions

Les *Discoglossidae,* Anoures très archaïques, sont, aujourd'hui, répandus en Europe, en Afrique du nord et en Asie orientale.

A l'état fossile, ils ne sont connus qu'en Europe. Le plus ancien qui ait été rencontré, *Prodiscoglossus Vertaizoni* Friant, vécut à l'Oligocène, en France (Limagne), non loin de l'Allier actuel. De grande taille et adapté au saut, il synthétisait, cependant, beaucoup de caractères des *Discoglossidae* vivants.

Puis apparaissent, au Miocène :

1°. Dans la Vallée du Rhin, des genres assez proches des actuels ou identiques à eux : *Discoglossus, Alytes, Pelophilus* (voisin de *Bombina*). L'un d'eux, *Alytes,* se maintient au Pliocène, dans le Bassin du Danube ; avec *Discoglossus,* il a persisté jusqu'à nous.

2° Dans la Vallée du Rhin également et aussi dans le Bassin de la Garonne, les géants du Groupe, dont l'un, *Latonia Seyfriedi* Meyer, est connu par de nombreux exemplaires. Outre leur très grande taille, ils étaint caractérisés par des ossifications dermiques au niveau du crâne, une mâchoire supérieure dentée « en peigne » et des membres postérieurs relativement courts. Ils s'éteignent au Miocéne supérieur.

Voici, schématissée, la répartition géologique des *Discoglossidae* d'Europe.

Actuel	*Discoglossus* *Bombina* *Alytes*	
Pliocène	*Alytes*	
Miocène	+ *Pelophilus* *Discoglossus* *Alytes*	+ *Latonia* + *Zaphrissa*
Oligocène	+ *Prodiscoglossus*	

La distribution géographique des *Discoglossidae* fossiles souligne qu'en Europe, dans les vallées des grands fleuves actuels et de leurs affluents, il y eut, à l'Oligocène, puis au Miocène et au Pliocène, de vastes lacs qui favorisèrent la vie des Batraciens.

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Nos connaissances paléontologiques prouvent que les *Discoglossidae* d'Europe ne semblent pas avoir une origine asiatique, comme, si longtemps, on l'a dit. Ces Anoures, déjà typiques à l'Oligocène, ont traversé les temps géologiques dans leur région d'origine, l'Europe, d'où *Discoglossus* a gagné l'Afrique et, sans doute, *Bombina,* l'Asie, à une époque encore inconnue de l'Histoire du Globe.

C'est un devoir pour moi de remercier, ici, très vivement M. M. les Professeurs H. Steiner et E. Kuhn, M. M. les Docteurs M. Schnitter et R. Handtke d'avoir mis à ma disposition, pour cette étude, toutes les ressources de l'Université et du Polytechnicum fédéral de Zürich.

Je remercie non moins vivement M. le Professeur B. Hanström de ses précieux conseils.

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Summary

The *Discoglossidae,* very archaic Anura, are living, today, in Europa, north Africa and east Asia.

At the fossil state, they are only known in Europa. The oldest, *Prodiscoglossus Vertaizoni* Friant, lived at the *Oligocene period* in France (Limagne), near the actual Allier. Though large and jumping, it synthetised many characters of the living *Discoglossidae.*

At the *Miocene period,* appear:

1°. In the Rhine Valley, some genera very near the actual ones or identical to them: *Discoglossus, Alytes, Pelophilus* (near *Bombina*). One of them, *Alytes,* is yet living during the Pliocene period, in the Danube Basin. With *Discoglossus,* it has persisted till our days.

2°. In the Rhine Valley and, also, in the Garonne Basin, lived the Giants of the group; one of them, *Latonia Seyfriedi* Meyer, is known by many specimens. They were very large Anura, with ossifications in the head's derm, superior teeth like those of a "comb" and short posterior legs. They disappear in the upper Miocene period.

The repartition of the fossil *Discoglossidae* underlines that, in Europa, along the great rivers' valley, during the Oligocene, Miocene and Pliocene periods, there were wide lakes, that favoured the Amphibian life.

The Palaeontology shows that the european *Discoglossidae* don't seem to have an Asiatic origin as, so often, it was said. Those Anura, typical in the Oligocene period, went, through the Geological times, in their original Land, from where *Discoglossus* reached Africa and, very probably, *Bombina,* Asia, at a period, yet unknown, of the Globe's history.

Madeleine Friant

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The development of the hypophysial vascular system in the lizards Lacerta a. agilis Linnaeus and Anguis fragilis Linnaeus and in the snake Natrix n. natrix (Linnaeus), with comparative remarks on the Amniota

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Introduction

Since Popa and Fielding (1930) discovered a portal system of vessels in the human hypophysis the hypophysial vascular system has been investigated in a fairly large number of adult animals, especially tetrapods. The hypophysial portal system is found to be of general occurrence. As to the lower vertebrates, however, our knowledge in this respect is still insufficient. The problems concerning the functional significance of the hypophysial portal system has called for intensified studies of the detailed arrangement of the vessels in the pituitary and adjacent parts of the hypothalamus.

Later on Wislocki and King (1936) stated that the blood stream in the portal vessels is the reverse of that supposed by Popa and Fielding, these contradictory views caused a scientific dispute extending over a considerable number of years. Today the views of Wislocki and King are almost generally accepted. The blood flows in the portal vessels towards the pituitary. The hypothesis that the vascular connection between the hypothalamic floor and the pars distalis of the pituitary means a possible way for the hypothalamus to control the activity of the pars distalis also caused intensive studies on especially the structure of the primary plexus of the hypophysial portal system. The said plexus may take up some humoral substances from the eminentia mediana, and these substances are then transported via the portal vessels to the pars distalis. Such a humoral transmission was first considered in detail by Harris (1944). Thus, due to the very important functional aspects paid to the hypophysial vascular system a large number of very good descriptions of the structure of the latter have accumulated during the two last decades.

The development of the hypophysial vascular system has not by far been studied to the same extent. Available information in this respect is restricted to a few mammalian species (*Homo* and a few rodents) and to a number of birds. The

existing literature will be dealt with in a later chapter. As regards the reptiles no investigation has so far been carried out on the development of the hypophysial vascular system, and the present work is the first attempt to fill this gap. Embryological studies may help to clear up the problems of the functional properties of a certain structure, but they are still more necessary when the morphological significance of the same structure should be clarified, for instance problems of homology etc. In the present paper the comparative view is in the first place applied to the study of the developing hypophysial vessels, and these comparisons are restricted to the *Amniota*. Such comparisons were carried out in an earlier paper (ENEMAR 1957), in which, however, only the conditions in *Aves* and *Mammalia* were discussed. Though the development in these classes showed the same course in the formation of the principal arrangement of the hypophysial vascular system, it also unveiled deviations in detail between the two classes which made an investigation on reptilian material desirable, especially as regards the venous system in the pituitary surroundings. In any case, as the reptiles are considered to have retained many primitive characters from the common amniotic stock, they should be studied before a general view on the course of the development of the amniotic hypophysial vascular system can be given.

The work was carried out at the Zoological Institute, Lund University. I wish to express my sincere thanks to Professor E. DAHL, Lund, and to Professor K. G. WINGSTRAND, Copenhagen, for good advice and for many discussions during my work. I also thank Mrs AGNETE VOLSOE for help with the correction of my English manuscript, and Dr. B. HYLTÉN-CAVALLIUS and Mr. S. ARLEBO for supply of material. The work has been supported by grants from the Swedish Natural Science Research Council.

MATERIAL AND METHODS

The choice of material was determined by the possibility to get a sufficient number of embryos from the few reptilian species living in southern Sweden. The common grass-snake, *Natrix n. natrix* (LINNAEUS), was first investigated, but since this species, like other members of the *Ophidia*, shows so many secondarily attained modifications in the anatomy of the head, e.g. lack of lateral lobes in the anlage of the adenohypophysis, asymmetric organization of the adult pituitary, a new side wall of the neurocranium formed by the parietal bones, and finally, laterial dislocation of the anterior portion of the vena capitis medialis during the embryonic development, it was desirable to include also other reptiles with a more primitive anatomy and embryology in these respects. Thus embryonic material also of the sand-lizard, *Lacerta a. agilis* LINNAEUS, and the slow-worm, *Anguis fragilis* LINNAEUS, was obtained and studied.

The descriptions of the embryonic vascular systems are based on preparations of ink-injected animals. When studying uninjected capillary plexuses or arrangements it is evidently very easy to overlook some of the vessels. Apparently, newly formed capillaries in the embryonic mesenchyme can easily collapse during fixa-

tion, and the paraffin embedding with overlooking as a consequence. Successful ink injections of course considerably reduce the risk of overlooking if the injection is complete. It is usually easy to state if this is the case or not. The injection technique may perhaps cause another error in the picture formed of the vascular arrangement if the ink is pressed out in the mesenchyme after rupture of a capillary. These artefacts are, however, easily recognized as they appear as diffuse ink-patterns in the mesenchyme and not as distinct tubes. When several embryos of each are injected and studied the error caused by the artefacts has practically no chance to influence the descriptions.

The embryos were injected according to the method described in my previous paper (ENEMAR 1957) by blowing the ink from the cannula via a rubber tube. It has been used with modifications by anatomists since the end of the nineteenth century. The injection cannula was always inserted in the ventricle of the beating heart of the embryo or the adult specimen. The earliest stages of *Anguis*-embryos were, however, injected via a vein in the area vasculosa. Before use the ink was filtered and then diluted to its double volume. Undiluted ink was also used occasionally.

The ink-injected material was fixed in alcohol-formol-glacial acetic acid (80 % alcohol, 90 cc/40 % formalin, 5 cc/glacial acetic acid, 5 cc) or in Bouin. The main part of the embryos was then embedded in celloidin and sectioned 60 μ—100 μ and mounted unstained or stained with Mallory's phosphostungstic acid haematoxylin (WINGSTRAND 1951 p. 15). Embryos at every stage were also embedded in paraffin, sectioned 6 μ—8 μ and stained by the azan method for the study of the morphogenesis of the hypophysis.

In a number of embryos of *Anguis* and *Lacerta* the ink-injected brain was dissected out from the brain-case, and the hypophysial region was exposed. These preparations were cleared in benzyl benzoate and used for microscopical studies of the arrangement of the vessels of the pituitary region in toto. This method proved to be the most suitable when studying the development of the primary plexus of the hypophysial portal system.

The embryonic material of *Lacerta* originated from seven egg-bearing females collected in a locality in southern Sweden. They were kept in a terrarium in the laboratory where they soon deposited their eggs in the sand. The earliest stages were obtained from the newly laid eggs. The eggs were incubated in an incubator and were kept in moist sand. The temperature was 35°—39° C. Hatching occurred after 23 days. Every three or four days some eggs were removed and their embryos prepared.

The main collection of *Anguis*-embryos was obtained from ten females, all found simultaneously in the same heap of grass and leaves. This species is viviparous. The specimens were kept in a terrarium in the laboratory, and every six days one or two induviduals were killed and dissected, and the embryos prepared. Fortunately, the embryonic development started at approximately the same time

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in all the females, and thus a fairly close-spaced series of embryos could be prepared. When the preparation started the embryos were already 33 mm long. The birth of the young took place five weeks later.

The eggs of *Natrix* were found on the same occasion in a dung-hill; there were three clutches of about the same size and age. The preparations started at once. After five days the eggs were removed and placed in an incubator at 30°—35° C together with some material from the finding-place. Hatching occurred four weeks after the collection of the eggs.

As a measure of the developmental stage the crown-rump length, CR, was obtained from the small embryos, and the total length, TL, with the tail included from the older ones. This is true for the embryos of *Lacerta* and *Anguis*. As regards *Natrix* the embryos were never stretched in order to be measured. It was obvious, however, that the developmental stage varied considerably in the embryos. Therefore, they were preliminarily arranged on the basis of the length of the liberated amnion sac with its embryo lying in its "natural pose". The definite classification of the *Natrix* embryos was based on the developmental stage of the hypophysial anlage. Six stages were selected, and they are described below in connection with the description of the morphogenesis of the hypophysis.

The material of embryos and adult specimens used in the present investigation is listed below. CR = crown-rump length, TL = total length, c = celloidin-sectioned, p = paraffin-sectioned, t = total preparation of hypothalamus cleared in benzyl benzoate, i = injected with indian ink.

Lacerta agilis: 6 adult specimens, 37 embryos.

CR 4 mm: 2 p; CR 5,5 mm: 3 ic, 2 p; CR 7 mm: 2 ic, 2 p; TL 28 mm: 2 ic, 2 p, 2 it; TL 33 mm: 2 it; TL 40 mm: 2 ic, 2 it; TL 45 mm: 1 it; TL 50 mm: 1 it; TL 55 mm: 2 ic, 2 it; TL 65 mm: 3 ic, 1 it; newly hatched (TL 65 mm): 3 ic; 2 days after hatching: 1 ic; adult: 2 ic, 1 ip, 3 it.

Anguis fragilis: 9 adult specimens, 106 embryos.

CR 2,5 mm: 2 ic, 5 p; TL 33—40 mm: 13 ic, 11 p, 10 it; TL 42 mm: 2 ic, 2 p; TL 46—54 mm: 4 ic, 3 p, 4 it; TL 60 mm: 4 ic, 2 p, 4 it; TL 62 mm: 3 ic, 3 p, 3 it; TL 70 mm: 6 ic, 3 p, 2 it; TL 73 mm: 4 ic, 2 p, 4 it; newly hatched (87—90 mm): 4 ic, 2 p, 4 it; adult: 3 ic, 1 ip, 2 p, 3 it.

Natrix natrix: 10 adult specimens, 73 embryos.

Stage I: 4 ic, 6 p; stage II: 14 ic, 10 p; stage III: 7 ic, 6 p; stage IV: 8 ic, 1 p; stage V: 6 ic, 1 p; stage VI (hatching stage): 7 ic, 3 p; adult: 3 ic, 1 ip, 3 p, 3 it.

NOMENCLATURE

In the present investigation the terminology given by WINGSTRAND (1951) is adopted. As regards the adult pituitary he used the nomenclature common in the Anglo-Saxon literature. Wingstrand thoroughly discussed the homologies of the hypophysis in the *Amniota* and, consequently, based his interpretation on em-

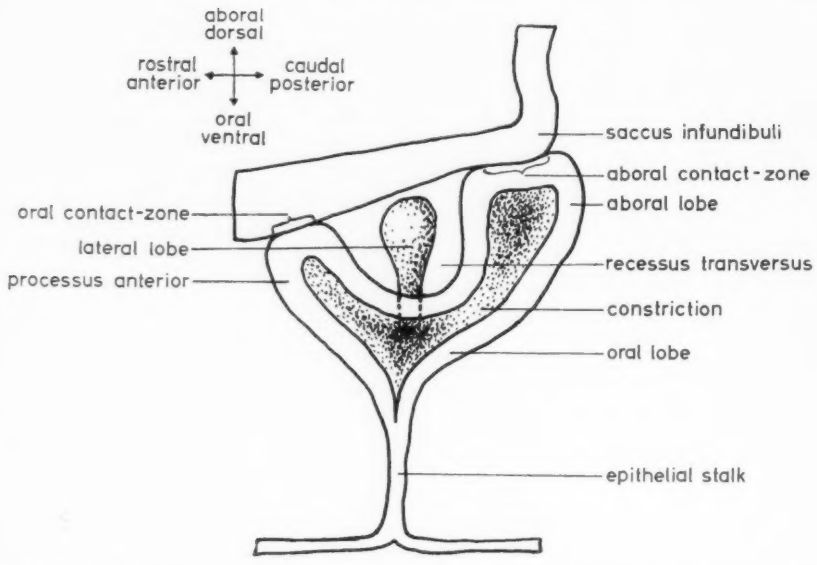

Fig. 1. Generalized diagram of the Rathke's pouch in the *Amniota* showing the terminology used in the present work. The diagram is redrawn and slightly modified after WINGSTRAND (1951, Fig. 41).

bryological studies of the organ; these studies involved a partly new nomenclature to be applied to the anlage of the amniotic hypophysis. The nomenclature has already been used by other investigators of the hypophysial development (SPRANKEL 1956, on the development of the hypophysis in *Chelonia*).

In order to simplify the description of the development of the hypophysis and its vessels Wingstrand's diagram of the hypophysial anlage in the *Amniota* has been redrawn and is shown in Fig. 1, with the nomenclature indicated.

The Rathke's pouch is the anlage of the adenohypophysis. The dorsal or aboral part of the pouch can be distinguished from the ventral or oral one, called the aboral lobe and oral lobe respectively. The boundary between the two lobes is often difficult to determine. The following criteria are useful when present: 1. The boundary is indicated by a constriction of the pouch, 2. the lateral lobes develop from the oral lobe near the boundary, and 3. the epithelial stalk is attached to the oral lobe. The oral lobe gives rise to the cephalic lobe of the pars distalis (except in *Mammalia*). The oral lobe often has a rostral prolongation, called the anterior process. The aboral lobe gives rise to the caudal lobe of the pars distalis in the adult. The most aboral portion of the aboral lobe often develops into the pars intermedia in the adult, which is in contact with the neural lobe.

In the description of the development of the vascular system the following

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concepts are useful: the oral contact-zone = the area where the anterior process of the oral lobe meets the brain surface; the aboral contact-zone = the area where the aboral lobe (the anlage of the pars intermedia) and the brain (the anlage of the neural lobe) meet; the recessus transversus = the mesenchyme-filled recess between the anterior process and the aboral lobe. The said contact-zones in the embryo partly correspond to the "Kontaktflächen" of Spatz which are described from adult mammalian pituitaries (cf. e.g. SPATZ 1954, 1955). The aboral contact-zone exactly corresponds to "die distale Adeno-neurohypophysäre Kontaktfläche" between the neural lobe and the pars intermedia in the adult. The oral contact-zone, i.e. the contact between the prospective eminentia mediana and the oral lobe of the adenohypophysial anlage, only exists during the embryonic life. The oral lobe when present withdraws from the brain wall and is replaced by the pars tuberalis in the contact-zone (although there are exceptions, e.g. *Lacertilia, Ophidia*). This contact between the pars tuberalis and the eminentia mediana in the adult is "die proximale Adeno-neurohypophysäre Kontaktfläche" of Spatz.

The lateral lobes (lobi laterales) are the anlage of the pars tuberalis in the adult. They have, however, often a peculiar development in the reptiles, and they are almost completely reduced in the *Ophidia*.

The saccus infundibuli is the anlage of the neural lobe and the infundibular stem. The part of the diencephalic floor located rostrally to the saccus infundibuli differentiates into the eminentia mediana. In the early embryos this area is indicated by the oral contact-zone.

Nomenclatural remarks on the vascular system are given in connection with the descriptions in the following chapters. Only the hypophysial portal system should be mentioned here. This concept comprises the vascular plexus on the eminentia mediana, called the primary plexus, which is drained by a bundle of portal vessels. The latter empty into the secondary plexus, which is the sinus-systems of the pars distalis.

The arteries supplying the primary plexus are called the infundibular arteries (a. infundibularis) in the present investigation and they correspond to the a. hypophysia superior in the human anatomy. The name "hypophysial artery" in this work is restricted to the arteries running directly to the pituitary, and when mentioned they generally correspond to the a. hypophysia inferior of the human hypophysis. It should be noted that the terms "oral" and "aboral" are used to denote directions towards the oral cavity and towards the brain respectively according to WINGSTRAND (1951). In the literature they have been used also in another sense, as e.g. in NEMEC (1952) where oral corresponds to rostral or anterior in Wingstrand's terminology and aboral denotes caudal or posterior.

The vascular supply

Under this heading the development of the hypophysial portal system and the vascular system of the neural lobe is described and discussed. Here also short descriptions of the morphology and development of the hypophysis in the investigated species are given. They are necessary as a back-ground to the study of the developing vascular pattern.

DESCRIPTIONS

Lacerta agilis

Notes on the morphology and vascular system of the hypophysis in the adult.

A more detailed investigation of the hypophysis in *Lacerta agilis* in both respects is not found in the literature. The adult morphology is described by KRAUSE (1921) and also by NEMEC (1952). STENDELL (1913) gives a picture of the organ and POKORNY (1926) included the species in his comparative study.

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The general shape of the hypophysis appears from Fig. 3. A median section showing the relation between the different lobes of the gland is seen in Fig. 2. The neurohypophysis which is located on the oral tip of the rather narrow and funnel-shaped tuber cinereum shows two thickenings in the median section. The first one is the eminentia mediana with the primary plexus of the hypophysial portal system. The eminentia is restricted to a comparatively small area on the oral wall of the tuber cinereum. The second is the neural lobe (lobus neurosus). The thin-walled tube between the eminentia mediana and the neural lobe is the infundibular stem. The third ventricle of the diencephalon extends down to the neural lobe where it ends in a more or less distinct bifurcation. The neural lobe thus has a primitive sac-like form and the said bifurcation is also visible on the surface of the lobe, since a deep median furrow divides the caudal part of the organ into two lateral portions.

The main mass of the adenohypophysis is located caudally to the neural lobe in the sella turcica and is caudally bounded by the dorsum sellae. Orally and laterally the pituitary is completely embraced by the retractor bulbi group of eye muscles. The pars intermedia of the adenohypophysis surrounds the neural lobe and consists of a relatively thin layer of cells. The pars intermedia caudally merges into the caudal lobe of the pars distalis via a relatively slender neck of the lobe. The rostral portion of the pars distalis, the cephalic lobe, extends rostrally to the infundibular stem and the caudal boundary of the eminentia mediana. The rostral portion of the cephalic lobe is narrow compared with the main bulk of the pars distalis and is separated from the surface of the infundibular stem and the eminentia mediana by a thin layer of connective tissue, corresponding to the

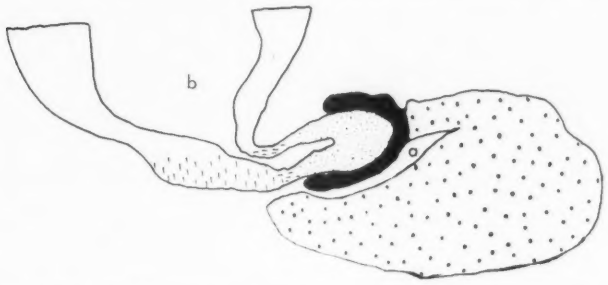

Fig. 2. *Lacerta agilis*. Adult. Camera lucida drawing of the median section of the hypophysis. Large dots = pars distalis and solid black = par intermedia of the adenohypophysis ; fine dots = neural lobe, horizontal short lines = infundibular stem, and vertikal short lines = eminentia mediana of the neurohypophysis. a = recessus transversus, b = third ventricle.

thick "pars terminalis" in the ophidian hypophysis. This connective tissue is continuous with that in the recessus transversus.

No pars tuberalis is found attached to the pars distalis, and the main portion of the eminentia mediana therefore is not covered by any glandular epithelium. The pars tuberalis is however not completely reduced but is found as cell masses included in the brain wall in the region of the sulcus infundibularis (sensu SPATZ, 1955) just above the caudal half of the eminentia mediana and near the base of the infundibular stem. NEMEC (1952) overlooked the existence of the pars tuberalis in this species and also in *Anguis fragilis*.

The vascular system of the hypophysis is supplied by the infundibular arteries only (Fig. 3). Each infundibular artery is divided into an anterior and a posterior ramus near its base on the ramus anterior of a. carotis interna. The anterior ramus of the infundibular artery runs to the region of the chiasma opticum. From here one or two thin branches curve caudally and run in the mid-line of the anterior wall of the tuber cinereum to the anterior border of the primary plexus on the eminentia mediana. The main tributary to the primary plexus is however the posterior ramus of the infundibular artery. The posterior ramus passes over the tuberal lobes before reaching the primary plexus. Both rami give rise to several arteries to the vascular system of the hypothalamus.

The primary plexus is a dense network of capillaries restricted in distribution to the eminentia mediana. The vascularization of the latter is enormous compared with that of the surrounding brain wall (Fig. 3). The vessels of the primary plexus do not form capillary loops in the tissue of the eminentia. They are thus restricted to the surface of the organ even if they are regularly situated in shallow furrows on it.

The capillary network on the infundibular stem and the neural lobe are continuous with the primary plexus. It is also strictly superficial. The neural lobe, however, forms numerous small secondary lobules and the capillaries are of course

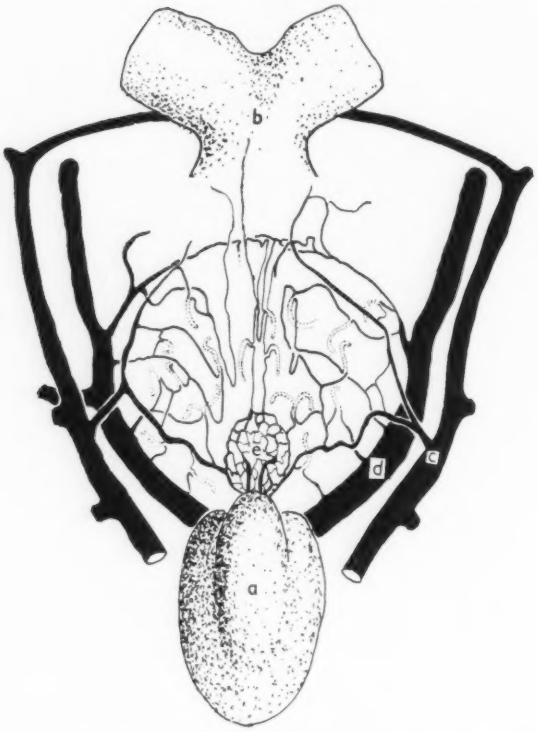

Fig. 3. *Lacerta agilis*. Adult. Camera lucida drawing of the configuration of vessels on the hypothalamus between the optic chiasma and the hypophysis. Oral view. Ink injection, cleared in benzyl benzoate. The course of the capillary loops penetrating the brain wall (dotted lines) are somewhat simplified in the drawing. — a = hypophysis, b = chiasma opticum, c = the anterior ramus of the internal carotid branching off the infundibular artery, d = vein draining the hypothalamus, e = primary plexus on the eminentia mediana (two portal vessels are visible).

included in the shallow furrows between the latter. The capillaries are never seen to penetrate the neural lobe tissue itself. A capillary plexus is always located to the deep median furrow of the neural lobe mentioned above. The main supply is managed by one or two twigs from the posterior ramus of the infundibular artery. They often run to the neural lobe on the aboral surface of the infundibular stem. Some blood also reaches the lobe via the capillaries connecting the primary plexus with the capillaries of the lobe. This communication is of course found on the oral surface of the infundibular stem.

The sinus-system of the pars distalis receives its blood from the portal vessels. The latter are few in number, four to six, and have their origin in the centre of the primary plexus, penetrate the thin connective tissue layer, and reach the cephalic lobe, where they immediately break up into the sinusoids of the lobe.

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No other supplying vessels to the lobe were found. The caudal lobe receives blood from the sinus-system of the cephalic lobe. A number of sinusoids in the latter have a rather straight course throughout the lobe, and they probably allow a relatively rapid transport of the blood through the cephalic lobe to the caudal one (Fig. 7).

The thin pars intermedia has no internal vascular system. Only superficial capillaries are found. At the margins of the lobe capillaries are seen to connect the vascular plexus of the neural lobe with that of the pars intermedia.

The vena retrohypophysea receives all blood leaving the hypophysial vascular system. The vein is a wide channel traversing the hypophysial region dorsally to the gland. It will be described in detail in connection with the description of its development.

The development of the hypophysis.

Members of the genus *Lacerta* have often been used by anatomists in their studies on the development of the hypophysis. Also the species used in this investigation was studied before in this respect (cf. HOFFMANN 1886, GAUPP 1893, BAUMGARTNER 1916) and the general outlines of the present description coincide with the results already published. The description below does not cover all aspects of the hypophysial development, but is concentrated to the main course of the developmental process and to such phenomena as are of special interest to the study of the embryology of the vascular system. These restrictions are also valid for the corresponding descriptions of the remaining species investigated in the present paper.

Embryo 4 mm CR. Fig. 4 A. The Rathke's pouch is in wide communication with the oral cavity. The epithelium of the pouch is thicker than the surrounding ephitelium of the oral roof. The primary contact between the anterior wall of the Rathke's pouch and the anlage of the infundibulum is locally broken by the mesenchyme invasion, and the cleft between the separated lamina of the two anlages is the recessus transversus. The contact between the Rathke's pouch and the brain persists orally and aborally to the recessus transversus in the so-called oral and aboral contact-zones. The first rudiments of the lateral lobes appear as small laterally directed buds. They are located to that part of the pouch where the recessus transversus is formed (see Fig. 4 A, b). Also at later stages they are located along the recessus transversus and thus constitute the lateral limitation of the latter. No evagination indicating the formation of the saccus infundibuli can be found at this early stage.

Embryos 5,5—7 mm CR. Fig. 4 B. During this period the distance between the brain and the oral roof becomes greater, and the hypophysial anlage is lengthened in an oral-aboral direction. The base of the Rathke's pouch is constricted and forms the epithelial stalk. The lumen of the pouch still communicates

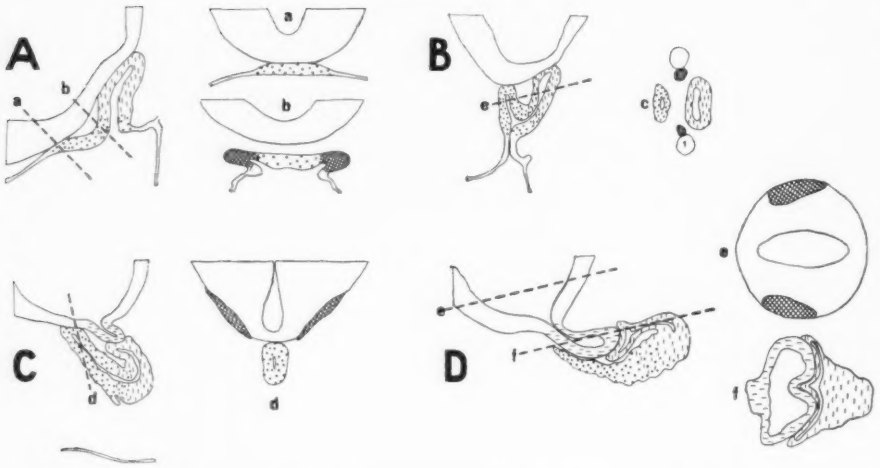

Fig. 4. *Lacerta agilis*. Camera lucida drawings of some stages of the development of the hypophysis. Each stage is represented by a median section, but in addition, drawings of one or two sections of different orientation are given. The section planes of the latter are approximately indicated by the broken lines in the drawing of the median section. Short vertical lines = aboral lobe if Rathke's pouch, dots = oral lobe of Rathke's pouch, crossed lines = lateral lobes, short horizontal lines = saccus infundibuli. 1 = the internal carotid. A = 4 mm CR, B = 7 mm CR, C = 28 mm TL, D = 55 mm TL.

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with the oral cavity via a very narrow tube in the stalk. The recessus transversus is deepened, and the median section of the pouch gives a U-shaped figure. The anterior leg of the U is the oral lobe of the hypophysial anlage and the posterior one the aboral lobe. Both lobes remain attached to the brain surface in the oral and aboral contact-zones. In this period of the development a secondary contact between the brain and the Rathke's pouch is established by the lateral lobes. They grow in an aboral direction and reach the brain between and laterally to the oral and aboral contact-zones. On their way to the brain the lateral lobes come into contact with the median wall of the internal carotids. The postoptic hypothalamus is a very deep and comparatively narrow funnel and remains so also in the adult stage. The saccus infundibuli has not yet appeared.

Embryo 28 mm TL. Fig. 4 C. At this stage the Rathke's pouch has begun to proliferate and to form epithelial cords giving rise to the glandular epithelium of the pars distalis. The epithelial stalk is now represented by a thin strand of cells or has partly disappeared. The distal ends of the lateral lobes have developed into a cell mass covering an area on the right and left wall of the tuber cinereum. These cell masses, which represent the definite anlage of the pars tuberalis in the adult, are situated aborally to the oral contact-zone (Fig. 4 C, second figure). These terminal parts of the lateral lobes have lost their primary connection with the proximal or basal part of the anlage. In some specimens a

thin strand of cells still unites the two parts of the lateral lobes.—A saccus infundibuli is formed from the brain wall in the aboral contact-zone. The said contact-zone is now consequently located to the tip of the saccus, and the adjacent part of the aboral lobe is the anlage of the pars intermedia. The whole hypophysial anlage is bent somewhat dorso-caudally. This dislocation is combined with changes in the common shape of the anlage. The oral and aboral lobes, the "legs" of the U, are pressed together, and the recessus transversus remains as a relatively narrow space between the lobes. The oral lobe is still in contact with the brain in the oral contact-zone.

Embryos 30—55 mm TL. Fig. 4 D. The hypophysis is now dislocated aborally into the plica encephali ventralis. The main axis of the anlage is almost parallel to the oral roof, a radical change from its original perpendicular orientation to the latter. The main part of the adenohypophysis is located caudally to the saccus infundibuli. The aboral tip of the aboral lobe has a relatively thin wall and covers the saccus to a certain extent and is identified as the pars intermedia. The oral half of the lobe and the oral lobe have proliferated and formed the glandular mass of the pars distalis. Their lumina have disappeared more or less completely. Since no traces of the proximal part of the lateral lobes or of the epithelial stalk are to be found the derivatives of the oral and aboral lobes, viz. the cephalic and caudal lobes of the pars distalis, cannot be separated with accuracy. Therefore, the boundary between the said lobes in characterized by a relatively broad transitional zone in the drawings. The pars tuberalis is represented by the two cell masses embedded in the surface of the brain. The oral lobe is still in a more or less close contact with the brain, but the contact-zone has moved caudally to the region of the base of the saccus infundibuli and sometimes covers part of the saccus too. This dislocation of the oral contact-zone is to be looked upon as a consequence of the moving of the whole hypophysial anlage as described above.—The saccus infundibuli is a thin-walled sac throughout. The base of the saccus is somewhat constricted indicating the anlage of the infundibular stem. The distal wall of the saccus curves inwards in the midline giving rise to the bilobed structure visible in a horizontal section of the organ.

Embryo 65 mm TL = the hatching stage. The final development until the hatching is of less importance in the present investigation and is mainly characterized by a general growth of the hypophysis. The distal part of the saccus infundibuli thickens and gives rise to the main mass of the neural lobe.

The development of the hypophysial portal system.

Embryo 4 mm CR. Rathke's pouch is encircled by a few capillaries. They are continuous with the capillary net-work on the adjacent brain wall. One or two capillaries also traverse the recessus transversus from one side to the other. The internal carotids pass close to the hypophysial anlage. It has not been pos-

sible to detect any arterial twigs from the carotids to the capillaries around the Rathke's pouch. Such arteries never seem to be developed in this species. The ramus anterior of the carotids branches off special vessel, the aa. infundibulares, at a level far aborally to the hypophysial anlage, and they supply the capillaries on the wall of the hypothalamus. Descending capillaries reach the tuber cinereum and the vessels surrounding the Rathke's pouch.

Embryos 5,5—7 mm CR. The number of capillaries in the mesenchyme around the lobes of the Rathke's pouch has increased. This is the case especially with the oral and aboral lobes. The lobi laterales, now extending to the brain, follow the internal carotids along the medial wall of the latter. They are not encircled by capillaries, at least not by far to the same extent as the oral and aboral lobes. After having passed the hypophysial anlage the internal carotids branch an artery to the eye anlage, the a. ophthalmica. They are then divided into the a. communicans posterior and the ramus anterior of carotis. The latter branches the infundibular artery. In one specimen a very thin intercarotic anastomosis was developed caudally and near the Rathke's pouch. This vessel, however, had no communication with the capillary plexus of the pituitary region.

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Embryo 28 mm TL. When the infundibular artery has left the ramus anterior of the carotid it runs rostro-orally and gives rise to capillary twigs supplying the capillary plexus of the brain wall. The course of the capillaries is relatively straight and orally directed. They finally converge to the oral contact-zone. About eight to ten straight capillaries reach in that way a vascular plexus in the oral contact-zone and empty there. This capillary congregation of the oral contact-zone is the anlage of the primary plexus. From here about four to five capillaries leave the brain surface and run orally in contact with and partly included in the proliferating epithelium of the oral lobe (Fig. 5). These capillaries from the oral contact-zone constitute the main supply to the vascular plexus of the whole adenohypophysial anlage, for at this stage only one or two scattered capillaries are found to leave the brain surface and run to the aboral lobe. The said vessels from the oral contact-zone are somewhat coiled and dilated and have a sinusoidal appearance. This is true also for the rest of the vascular plexus on the oral and aboral lobes, and the vessels are all included in the epithelial masses, thus giving rise to the sinus-system of the growing pars distalis. Contrary to the vessels on the hypophysial anlage the straight capillaries on the brain wall are all very thin.

In this connection it should be mentioned that the origin of the vascular system of the pars distalis in *Lacerta agilis* was commented upon already by Gaupp (1893). According to this author capillaries surrounding the hypophysial anlage are of a venous nature, an opinion contrary to that of earlier investigators who erroneously considered the capillaries as branches from the internal carotids.

Some straight capillaries on the brain wall pass the cell masses of the lateral lobes on their way to the oral contact-zone. This passage is located to the surface of the epithelial masses, not between the latter and the brain wall. It should also

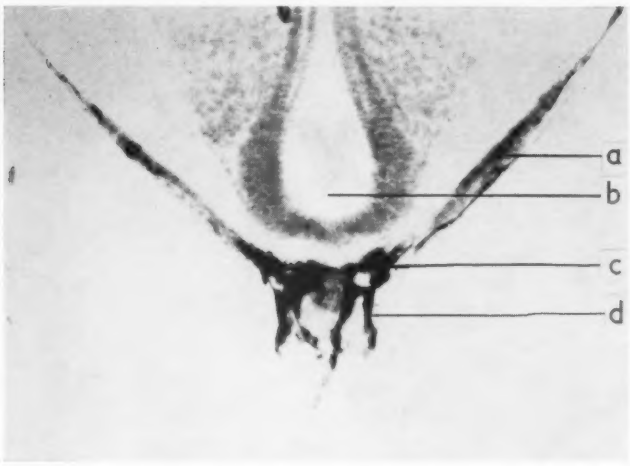

Fig. 5. *Lacerta agilis*. Embryo 28 mm TL. Transverse section through the oral contact-zone of the hypophysial anlage showing the capillaries descending from the plexus of the contact-zone into the adjacent part of the oral lobe. — a = cell mass of the lateral lobe on the surface of the tuber cinereum, b = the third ventricle, c = the capillary plexus of the oral contact-zone, d = one of the vessels connecting the vascular plexus of the oral contact-zone with that of the oral lobe. — Ink injection, celloidin 75 μ, Mallory's phosphostungstic acid haematoxylin, blue filter, 170 ×.

be noted that no vessels use the mesenchyme-filled recessus transversus as a path from the oral contact-zone to the hypophysial anlage.

Embryo 33 mm TL. Judging from two ink-injected and unsectioned preparations of the hypophysis and adjacent region it is evident that the development of the primary plexus has already reached a relatively advanced stage (Fig. 6 A). The congregation of capillaries in the oral contact-zone mentioned in the previous stage has differentiated into a very dense net-work comprising about thirty meshes, that is approximately as many as in the adult primary plexus. It is, however, not so distinctly separated from the capillary pattern of the surrounding brain wall as in later stages and in the adult. The straight vessels running to the oral contact-zone has partly disappeared, but some of them have begun the transformation into the main supplying vessels of the hypophysis in the adult. As a result of this process the infundibular artery is near its base represented by two main branches. The anterior branch runs rostrally towards the chiasma opticum and gives rise to several branches to the brain in this region. Near the chiasma it suddenly curves caudally and runs near the midline and often in company with the corresponding vessel of the opposite side and finally empties into the primary plexus at the anterior border of the latter. The posterior ramus runs directly to the base of the saccus infundibuli and always seems to pass over the cell masses of the tuberal lobes. It gives rise to a few branches to the tuber cinereum

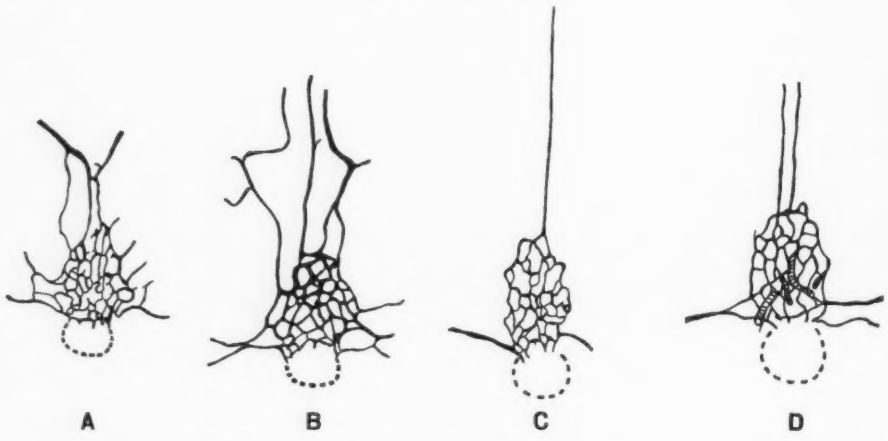

Fig. 6. *Lacerta agilis*. Camera lucida drawings of four stages of the primary plexus, showing how it is separated from the numerous communications with the vascular plexus of the adjacent brain wall. The portal vessels are marked out only in the most advanced stage (the thick banded vessels). The broken line indicates the position of the saccus infundibuli. A = 33 mm TL, B = 40 mm TL, C = 50 mm TL, D = 65 mm TL (hatching stage). The figures are drawn from dissected brain anlages of embryos injected with Indian ink.

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and to the primary plexus and also constitutes the supply of the saccus infundibuli.

The oral lobe of the hypophysial anlage still reaches the brain wall in the oral contact-zone and thus covers the main part of the primary plexus. The vessels connecting the primary plexus with the secondary plexus—the latter being the vascular system of the growing pars distalis—rise from a central area of the primary plexus.

Embryos 40—55 mm TL. The anlage of the pars distalis grows much during this period. The primary plexus becomes more distinct from the capillary pattern in the surroundings, and at the end of the period it is bordered by a marginal capillary (Fig. 6 B, C). The oral lobe is still in contact with the primary plexus in the 40 mm and 42 mm stages, but later this primary contact is broken. This happens in connection with the caudal dislocation of the hypophysial anlage as described above (p. 153). As a result of this process the end of the oral lobe is separated from the primary plexus on the eminentia mediana. However, the primary and secondary plexuses still communicate, and the linking vessels are the portal vessels. The forerunners of the latter are the earlier described capillaries which connected the vascular system of the brain wall with that of the hypophysial anlage. In the 40 and 42 mm stage these linking capillaries are seen to have thickened and they are comparatively few, four or five in number. When the oral lobe has moved away these vessels bridge the gap between the eminentia mediana and the lobe. These unbranched and relatively wide-spaced vessels be-

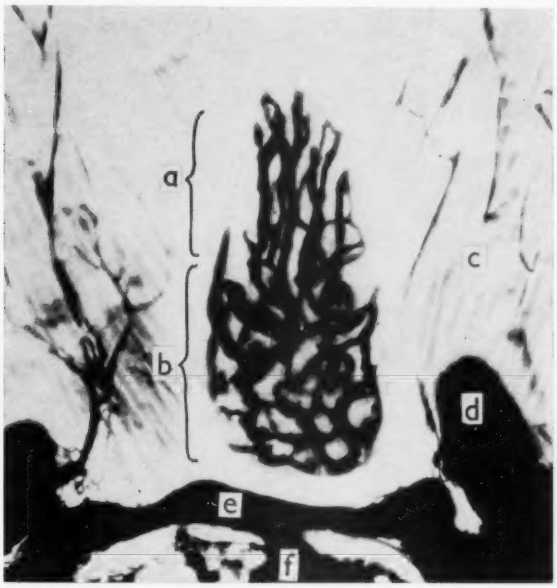

Fig. 7. *Lacerta agilis*. Newly hatched. Horizontal section through the oral half of the sella showing the pattern of sinusoids in the cephalic (oral) lobe and caudal (aboral) lobe of the pars distalis. — a = the cephalic lobe with its relatively straight sinusoids, b = the caudal lobe with a more net-like configuration of sinusoids, c = muscles of the retractor bulbi group, d = the internal carotid, e = the vena intrasellaris transversus, f = blood caverns in the base of the dorsum sellae in communication with the vena intrasellaris transversus. — Ink injection, celloidin 75 μ, Mallory's phosphostungstic acid haematoxylin, blue filter, 165 ×.

tween the two plexuses may be designated as true portal vessels. As soon as they have reached the lobe they branch into a number of sinusoids. The course of the latter is relatively straight in the rostral half of the oral lobe, and some of them continue their straight course on to the aboral lobe, thus appearing as intralobular continuations of the portal vessels. The complicated pattern of sinusoids is established in the aboral lobe and in the caudal half of the oral lobe (Fig. 7).

Embryo 65 mm TL (the hatching stage)—adult. The vascular organization attained at the end of the previous period is maintained into the adult stage. The number of meshes in the primary plexus is much the same (30—40 in number) and the portal vessels, always leaving the centre of the primary plexus, are few, only three to five in the investigated specimens (Fig. 6 D). The pars intermedia remains thin, and it is vascularized by a superficial network of capillaries. This network is also found between the intermedia and the neural lobe.

Anders Enemar

The development of the vascular system of the neural lobe.

When the saccus infundibuli is distinct in the 28 mm embryo it is surrounded by capillaries which are continuous with the capillary bed of the adjacent brain wall and with the vessels surrounding the Rathke's pouch. The mesenchyme has invaded the space between the saccus and the aboral lobe in the aboral contact-zone, and this invasion is combined with the appearance of capillaries in the same space. The capillaries on the saccus infundibuli are of course connected with those of the oral contact-zone, and this primary communication between the two capillary beds is maintained in the following development and in the adult.

In the following stages to the 55 mm stage the vascular plexus of the saccus infundibuli becomes a denser network of relatively thin capillaries. The saccus is completely encircled, and the vessels also invade the narrow space between the two distal lobes of the saccus. Several capillaries also link the vessels of the saccus infundibuli and those covering the outer surface of the anlage of the pars intermedia.

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The relatively dense network of the saccus infundibuli also includes the infundibular stem when the latter becomes distinct. It is in fact not possible to state the exact caudal border of the primary plexus as it gradually merges into the capillary network of the oral surface of the infundibular stem. As previously mentioned (p. 155) the posterior ramus of the infundibular artery constitutes the main supply to the saccus, and it terminates in the capillary bed at the base of the infundibular stem. The portal vessels leave the primary plexus some distance anterior to this point. It is of course possible that part of the blood content of the primary plexus takes its way via the capillaries to the saccus infundibuli. These linking capillaries are, however, very thin compared with the portal vessels, and therefore it may be concluded that the main blood stream from the primary plexus is destined for the pars distalis via the portal vessels.

Beside the portal vessels and the capillaries between the saccus infundibuli and the pars intermedia there are no vascular connections between the neural and epithelial parts of the hypophysial anlage. No vessels traverse the narrow recessus transversus which is filled with connective tissue and thus separates the vascular systems of the oral lobe on one hand and of the saccus and the pars intermedia on the other.

In the following development until hatching and thereafter to the adult stage the vascular arrangement just described is preserved. The distal wall of the saccus infundibuli thickens considerably and forms the neural lobe, but it is never vascularized in its internal parts. Thus the vascular system of the whole neurohypophysis is strictly superficial throughout.

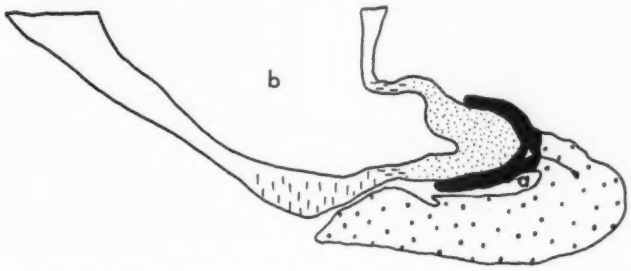

Fig. 8. *Anguis fragilis.* Adult. Camera lucida drawing of the median section of the hypophysis. For explanations see Fig. 2.

Anguis fragilis

Notes on the morphology and vascular system of the hypophysis in the adult.

The literature dealing with the hypophysis in *Anguis* is meagre. Comments on its morphology and vascular system are given by DIEPEN (1955), but they only concern the relation eminentia—pars distalis. WINGSTRAND (1951, p. 142, 128) has also contributed to the characterization of the morphology of the gland. NEMEC (1952) describes the main organization of the pituitary in a young, newly hatched specimen, and POKORNY (1926) included the species in his comparative work.

The general morphology of the hypophysis in *Anguis* (Fig. 8) is similar to that of *Lacerta agilis* and therefore it will only be briefly discussed here.

The neural lobe is a sac of a simpler construction than that of *Lacerta.* It consists of the thick walls of the distal part of the embryonic saccus infundibuli and does not show furrows or secondary lobules on its outer surface. The lumen of the lobe is in broad communication with the third ventricle, and the infundibular stem is not a stem in the sense of a relatively narrow connection between the neural lobe and the eminentia mediana. It can only be identfied as a very thin-walled portion of the saccus.

The thin pars intermedia shows its original double-layered condition, and the space separating the two layers of the lobe can sometimes be followed down into the caudal lobe. This space is a rudiment of the lumen of the Rathke's pouch. The pars distalis and the pars tuberalis have the same organization as in *Lacerta.* It seems to be a constant difference, however, that the pars distalis is not dislocated so far caudally in *Anguis* as in *Lacerta.* Therefore, the cephalic lobe always covers a large part of the eminentia mediana in *Anguis.* Also in this species a thin layer of connective tissue separates the pars distalis from the brain wall of the oral contact-zone, and it is likewise continuous with the connective tissue in the narrow recessus transversus.

The hypophysis is located in a shallow sella turcica as in *Lacerta.* A difference

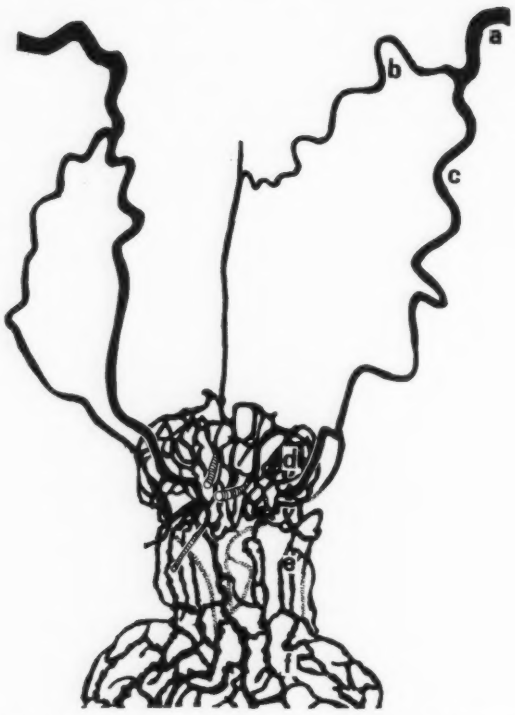

Fig. 9. *Anguis fragilis*. Adult. Camera lucida drawing showing the communication between the primary plexus and the vascular system of the neural lobe. — a = infundibular artery, b = its anterior ramus, c = its posterior ramus, d = the primary plexus with four portal vessels (denoted as striated vessels), e = the relatively straight capillaries of the infundibular stem, mainly located to its oral surface; the capillaries on the aboral surface of the infundibular stem are marked as strands of dots, f = the capillary plexus of the rostral half of the neural lobe. — Ink injection, cleared in benzyl benzoate. Pars distalis is removed. Oral view.

is that the retractor bulbi group of eye muscles do not invade the sella in *Anguis*. The muscles are inserted to the ventral edges of the foramen metopticum just laterally to the hypophysis.

The hypophysial vascular system is supplied by the infundibular arteries only. They are often divided into an anterior and a posterior ramus as in *Lacerta* although irregularities may be found. The posterior ramus constitutes the main tributary to the hypophysial portal system, but the anterior branch also takes part in it, often by way of a single median straight vessel from the neighbourhood of the chiasma as in *Lacerta* (Fig. 9). The supplying vessels terminate in the primary plexus on the eminentia mediana. The plexus is a very dense net-work of capillaries which has only occasionally a capillary communication with the capillaries in the surrounding brain wall. The primary plexus shows no loops into the

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eminential tissue, but is strictly located to the surface of the organ partly in very shallow furrows of the latter. It is drained by a few portal vessels, usually four or five in number, which penetrate the connective tissue covering the eminentia and reach the cephalic lobe where they join the sinus-system. The caudal lobe is supplied via the sinus-system of the cephalic lobe in a way similar to that in *Lacerta*.

The primary plexus is also drained towards the neural lobe. Its capillaries continue over the oral surface of the infundibular stem (Fig. 9) as a number of relatively straight and unbranched vessels. When reaching the neural lobe they join the capillary net-work on the surface of the latter. The neural lobe lacks lobules and furrows on its surface, and therefore its vascular system appears as strictly superficial. The lobe is also supplied by a few vessels running on the aboral surface of the infundibular stem. One of these vessels can take its origin in the posterior ramus of the infundibular artery and then acts as a special tributary to the neural lobe, completely avoiding the primary plexus. Several communicating capillaries are found between the capillary plexuses of the neural lobe and of the pars intermedia.

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The development of the hypophysis.

GAUPP (1893) described the anlages of the different lobes of the hypophysis in *Anguis*, and WINGSTRAND (1951) has taken up some stages of the development in his comparative discussions (op. cit. 127—128). A coherent description of morphogenesis throughout was not found in the literature.

E m b r y o 2,5 m m C R (after fixation). Fig. 10 A. At this stage that part of the oral epithelium which belongs to the Rathke's pouch could not be delimited with certainty. The epithelium forms an angle in the median section, and this angle is the beginning of the formation of the adenohypophysial anlage. The tip of the chorda is attached to the tip of the angle (cf. OPPEL's descriptions (1890) of this region in corresponding stages in *Anguis*). The anterior wall of the prospective Rathke's pouch is attached to the brain anlage throughout, and it is not possible to distinguish the epithelium of the hypophysial anlage from that of the remaining oral cavity. There is no indication of a saccus infundibuli.

E m b r y o s 3 3—4 2 m m T L. Fig. 10 B. In the 33 mm embryo the hypophysial anlage has reached a relatively advanced stage. Unfortunately, no embryos in stages between 2,5 mm CR and 33 mm TL were available for investigation. The intervening stages are probably similar to the corresponding ones in *Lacerta agilis*, at least judging from the common similarity in the development of the later stages. This interval in the series, however, does not considerably disturb the study of the development of the hypophysial vessels.

The epithelium of the Rathke's pouch is almost completely constricted off from the oral roof. A string of cells still connects the hypophysial anlage with the oral

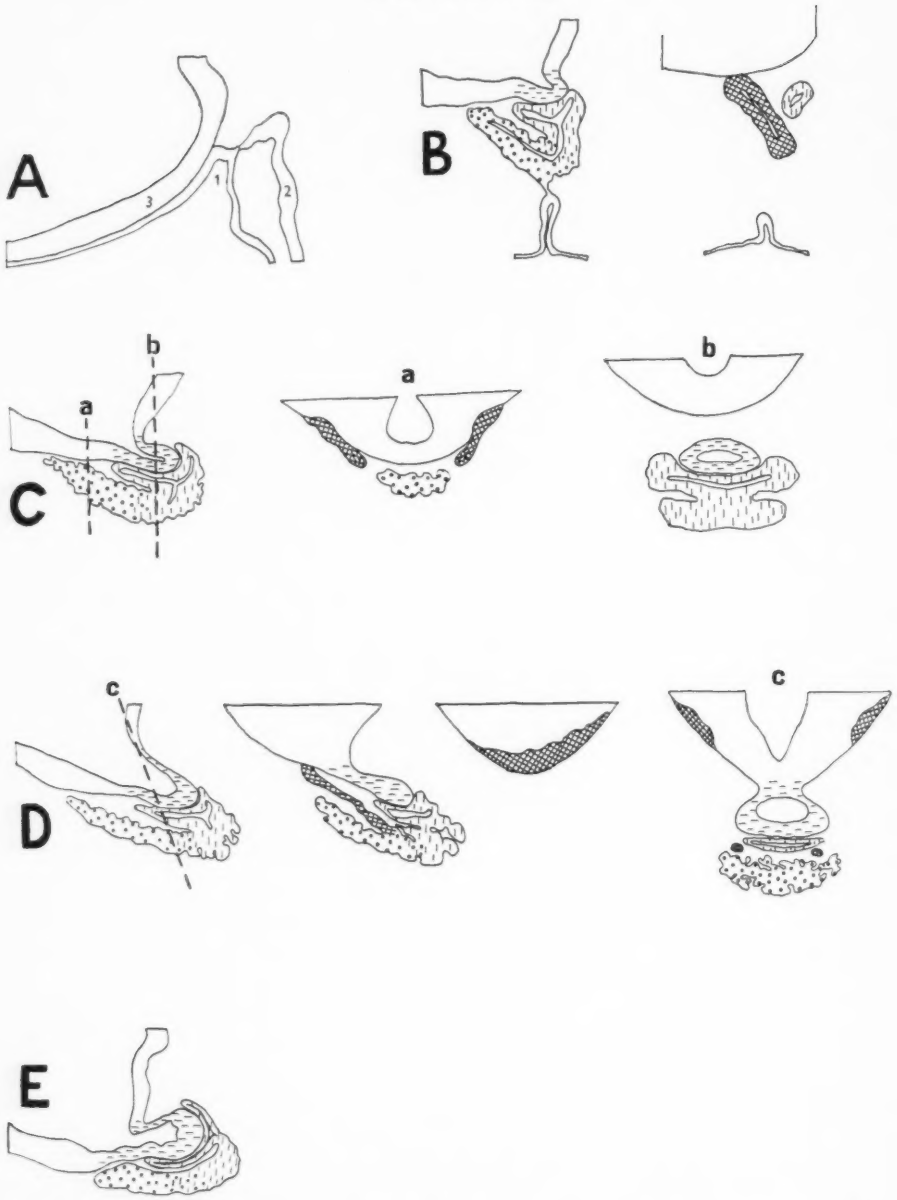

Fig. 10. *Anguis fragilis*. Camera lucida drawings of some stages of the development of the hypophysis. A = 2,5 mm CR. Median section. 1 = Rathke's pouch, 2 = chorda, 3 = brain wall. B = 33 mm TL. The left figure is a median section, right figure a paramedian section of the same specimen at the level of the lateral lobe. C = 46 mm TL. Median section and two transverse sections. The positions of the latter are indicated by the broken lines in the median section. D = 60 mm TL. To the left a median section and two successive paramedian sections, the latter showing the extension of the lateral lobes. To the right a transverse section, the section plane of which is marked out in the median section. E = hatching stage. Median section. For explanations, compare also Fig. 4.

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epithelium. This epithelial stalk is very slender nearest the anlage of the hypophysis, but its oral part has a considerable lateral extension. A transverse section through the embryo at the level of the epithelial stalk shows a triangular epithelial plate with the base on the oral roof. From the tip of the plate a string of cells can be followed to the hypophysial anlage. The double-layered epithelial plate also shows a swelling in the median section as seen in Fig. 10 B.

The Rathke's pouch has formed the main lobes of the hypophysial anlage. The oral and aboral lobes are easily identified. A considerable amount of mesenchyme is found between the oral lobe and the brain wall in the oral contact-zone, especially in a median area of this zone. The transverse recess is a fairly narrow space between the oral and aboral lobe. In the aboral contact-zone a saccus has developed. The epithelium of the two lobes of the Rathke's pouch has begun to proliferate, but their lumina has not yet disappeared. Two large lateral lobes are found as hollow buds reaching the brain surface. Their distal ends on the brain has started to proliferate in the same manner as in *Lacerta*. As seen in Fig. 10 B the lateral lobes constitute a prominent structure in this reptilian species and are of the same size as the oral lobe.

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Embryos 46—54 mm TL. Fig. 10 C. During this period the main organization of the hypophysial anlage reaches that of the pituitary in the adult. The whole anlage is pressed somewhat in an aboral direction. This process seems, however, not to be combined with a moving of the epithelial anlage from the primary position orally to the saccus infundibuli to a position caudally to the saccus to the same extent as in *Lacerta*. The oral lobe (more strictly the processus anterior of the oral lobe) is therefore still in contact with the brain surface in the region of the prospective eminentia mediana. The adenohypophysial anlage shows an intensive proliferative activity, and the lumen of the oral lobe has disappeared. The aboral part of the aboral lobe is still thin-walled and contains a lumen which persists in the adult gland. It is attached to the saccus infundibuli and forms the thin pars intermedia. The epithelial stalk is reduced, and no remains can be detected on the hypophysial anlage. The lateral lobes are still comparatively large, and they reach with their distal cell masses relatively high up on the wall of the tuber cinereum. Near the oral lobe the lateral lobes are tubular, thus retaining their original lumen.

Embryos 60—62 mm TL. Fig. 10 D. There are only few changes in comparison with the preceding stage. The dislocation of the epithelial anlage has progressed, and the main part of the aboral lobe is located caudally to the saccus infundibuli. The oral lobe is stretched, but the processus anterior still reaches the region of the prospective eminentia mediana. The lateral lobes are long and slender structures which run from the anlage of the pars distalis to the cell masses on the brain. These cell masses have proliferated in the last stages, and they now cover a considerable area on the lateral walls of the tuber cinereum (Fig.

10 D). The wall of the distal part of the saccus infundibuli has begun to thicken and form the neural lobe.

Embryos 70—90 mm TL (hatching stage). Fig. 10 E. A neural lobe is developed, but this structure is still sac-like with a wide lumen. That part of the saccus infundibuli which forms the tissue of the neural lobe is covered by a thin double-layered pars intermedia. The space between its laminae is the rest of the lumen of the Rathke's pouch. Part of the floor of the tuber cinereum differentiates into the eminentia mediana and between this structure and the neural lobe the infundibular stem is found. The oral lobe is in contact with the oral surface of the infundibular stem, and also reaches to the caudal part of the eminentia mediana. The cell masses on the brain wall, the pars tuberalis, are included in the brain wall in a groove situated dorso-laterally to the eminentia. At the hatching stage the communicating cell strings between the pars tuberalis and the pars distalis are broken in most cases. The recessus transversus is a very narrow mesenchyme-filled space between the oral lobe and the oral surface of the pars intermedia.

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The development of the hypophysial portal system.

Embryo 2,5 mm CR. The vascularization of the brain anlage is poor. The internal carotids touch the lateral walls of the Rathke's pouch when passing the pituitary region. They are also in contact with the oral wall of the praemandibular cavities. In one injected specimen a very thin anastomosis between the carotids traverses the pituitary region at the tip of the Rathke's pouch. Otherwise no capillaries are found in the mesenchyme surrounding the hypophysial anlage.

Embryos 33—37 mm TL. This stage is considerably later than the preceding one, and the characteristic embryonic vascular pattern of the developing hypophysis is formed (Figs. 11 and 12). The internal carotids pass the lateral walls of the Rathke's pouch and then curve aborally. Near the aboral limit of the plica encephali ventralis they divide into the posterior communicating arteries and the ramus anterior of the carotid. Then the latter is divided into one branch to the mesencephalon, and another branch which curves rostrally and supplies the area of the telencephalon. Near the said dividing point the anterior artery gives rise to a vessel which runs orally on the diencephalic wall. This is the infundibular artery (Fig. 12). Having reached the hypothalamus this artery is split up into the capillary network. This network is very dense on the rostral brain wall near the chiasma opticum. From this region and orally down to the hypophysial anlage the capillaries on the brain surface do not anastomose to the same extent, but run as relatively straight and unbranched capillaries and converge to the oral contact-zone of the hypophysial anlage (Fig. 12). These straight capillaries were counted in several injected specimens, and their number seems to be about ten to twelve. In the oral contact-zone they anastomose, and from there

Fig. 11. *Anguis fragilis*. Embryo 33 mm TL. Median section of the hypophysial anlage showing the vessels running from the brain surface of the oral contact-zone along the processus anterior of the oral lobe. — a = the oral contact-zone, b = recessus transversus, c = the processus anterior of the oral lobe, d = the epithelial stalk, e = saccus infundibuli, f = the anlage of the pars intermedia, g = the aboral lobe of the Rathke's pouch, h = the anlage of the vena intrasellaris transversus. — Ink injection, celloidin 60 μ, Mallory's phosphostungstic acid haematoxylin, blue filter, 145 ×.

a bundle of capillaries—five to six in number—run along the oral lobe of the hypophysial anlage and join the capillary network which at this stage surrounds the hypophysial anlage. The capillaries on the oral lobe are partly included in the proliferating epithelium. It should be noted that the vessels from the brain wall to the hypophysial anlage all run in contact with the oral lobe (processus anterior), and that no vessels are found in the mesenchyme of the recessus transversus (Fig. 11). The main supply of the aboral lobe seems to be via the dilated vessels of the oral lobe, but a few capillaries may also be found running from the brain wall to the aboral lobe in the aboral contact-zone. It should also be stressed that the lateral lobes are not used as a bridge for the capillaries between the anlages of the eminentia and the adenohypophysis. Only occasionally were a few thin capillaries found on the surface of the lateral lobes.

No intercarotic anastomosis and no arterial twig from the internal carotid to the adenohypophysial anlage were found in the examined embryos.

Embryos 42—54 mm TL. During this period the epithelium of the

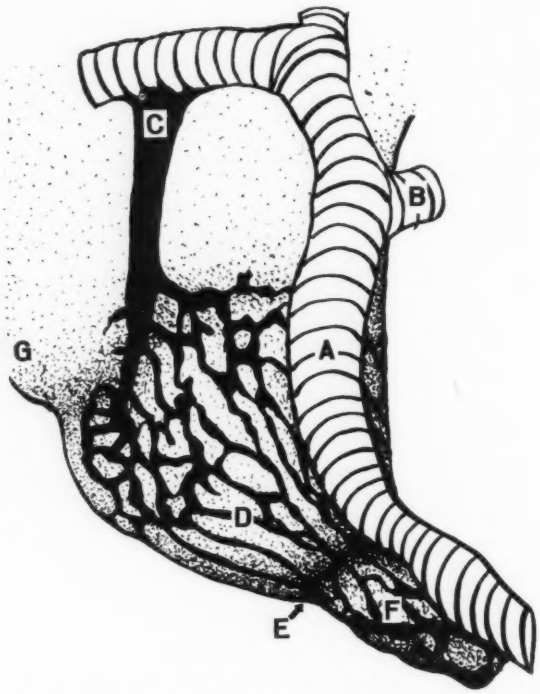

Fig. 12. *Anguis fragilis*. Embryo 33 mm TL. Camera lucida drawing of the anlage of the hypothalamus showing the arrangement of straight capillaries converging towards the oral contact-zone. Lateral view. A = a. carotis interna, B = a. communicans posterior, C = a. fundibularis, D = straight capillaries, E = the oral contact-zone, F = the oral lobe of the adenohypophysial anlage (the rest of the anlage of the hypophysis is hidden behind the carotid), G = the region of chiasma opticum. — Ink injection, cleared in benzyl benzoate.

Rathke's pouch is rapidly proliferating and forming the main bulk of the pars distalis. The capillary plexus surrounding the anlage is included among the cell cords, and thus the secondary plexus of the hypophysial portal system is established. However, the lateral lobes and the anlage of the pars intermedia do not show this activity, and they do not establish an internal vascular system. The lateral lobes have now given rise to the cell masses from their distal ends on the surface of the brain. These masses do not take up and embrace any capillaries, and no capillaries are found between the cell masses and the brain wall. The mesenchyme together with vessels now invade the space between the intermedia anlage and the saccus infundibuli in the aboral contact-zone.

A characteristic alteration in the vascular pattern of the brain wall has occurred in comparison with the preceding stage. The number of straight capillaries converging to the oral contact-zone is reduced in all embryos investigated, and their number is now four to seven. One or two median capillaries in the

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primary set of straight capillaries are preserved, and also a pair of the more laterally running capillaries on each side. These remaining median and lateral capillaries form the definite vascular supply to the primary plexus and the neural lobe in the adult animal. In the oral contact-zone the anastomosing capillaries mentioned in the previous stage are retained and they constitute the anlage of the primary plexus. The network of this anlage seems now to be more advanced in extension and number of meshes compared with the previous stage, but it has not yet the full characteristic organization of a primary plexus. From the capillary network in the oral contact-zone capillaries of course run to the oral lobe. Their number has been estimated at seven or eight. These vessels supply the oral and aboral lobes. The latter may also be supplied by a few capillaries connecting the vascular plexus of the saccus infundibuli and that of the aboral lobe. It is however not possible to ascertain the direction of the blood stream in these vessels.

Embryos 60—62 mm TL. At this stage the capillary plexus of the oral contact-zone has developed into a characteristic primary plexus consisting of a distinctly delimited area covered by a dense network of capillaries separated from the few superficial capillaries on the adjacent brain wall. The primary plexus is supplied by a few thin arteries, the forrunners of which were described in the preceding stages, viz. one lateral artery from each side being the posterior ramus of the infundibular artery and one or two median vessels from the superficial capillary plexus near the chiasma opticum. The formation of the portal vessels has apparently begun at this stage. The oral lobe is not removed from the anlage of the eminentia, and consequently there is a relatively close contact between the primary plexus and the distal termination of the lobe (the processus anterior). The latter is excavated, and the resulting shallow excavation is filled with connective tissue on to the brain wall. This layer of connective tissue is penetrated by the vessels running from about the centre of the primary plexus to the secondary plexus. They are dilated compared with the vessels of the primary plexus, and they are only four to five in number. In other words, they show the qualities of the portal vessels in the adult animal. They are however very short and soon split up into the sinusoids of the oral lobe.

The tuberal lobes are now very extended and are included in the brain wall. The posterior rami of the infundibular arteries take their way over the tuberal lobes and terminate in the caudal portion of the primary plexus exactly in the same way as in *Lacerta*. No capillaries are included in the tuberal lobes.

Embryos 70—73 mm TL. The oral lobe is slightly removed from the eminentia in the caudal direction, and the enlarged gap between the lobe and the eminentia is filled with a conspicuous mass of connective tissue. The portal vessels are embedded in this tissue (corresponding to the pars terminalis in the snakes) as in the previous stage, but they are now easier to distinguish, still about five in number. The hypophysial portal system thus has its definite characteristics

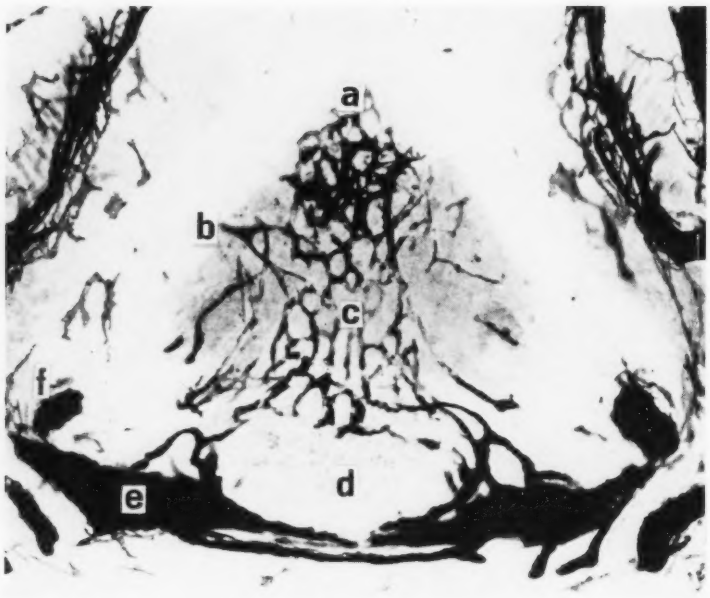

Fig. 13. *Anguis fragilis*. Embryo 73 mm TL. Horizontal section including the neurohypophysis. Oral view. — a = the primary plexus with a few cut portal vessels, b = the posterior ramus of the infundibular artery joining the caudal border of the primary plexus, c = the capillary plexus on the prospective infundibular stem, d = the saccus infundibuli, the anlage of the neural lobe, e = the vena retrohypophysea, f = the internal carotid. — Ink injection, celloidin 60 μ, Mallory's phosphostungstic acid haematoxylin, blue filter, 150 ×.

from now on, consisting of a primary and secondary plexus well separated from each other, but communicating via a bundle of short but relatively wide portal vessels. The tributaries to the primary plexus are the same as in the preceding stage. The vascularization of the brain proper has of course increased during the development. This process is manifested in the development of capillary loops deeply penetrating the brain tissue from the superficial plexus of vessels. At the present stage such loops are sometimes seen to pass the tuberal lobes from their surface into the brain tissue, but this does not mean that a special vascular plexus of the tuberal lobes is established. The primary plexus remains superficial.

Embryos 87—90 mm TL (the hatching stage). Apart from the general growth of the hypophysis the only change from the preceding stage is that the adenohypophysis has moved a little further in the caudal direction. The rostral end of the lobe is now located to the height of the infundibular stem. Consequently the portal vessels all leave the primary plexus in front of the oral lobe and run the short distance in the connective tissue and there join the epithelial mass of the oral lobe. Therefore, it is possible to count the number of meshes in that part of the primary plexus which surrounds the base of the portal vessels.

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It seems to comprise about 20 meshes in the network. It is however not possible to count the meshes exactly as the primary plexus is not distinctly delimited caudally where it is continuous with the capillaries on the oral surface of the infundibular stem (Fig. 13). The number of meshes of the primary plexuses in the previous stages seems to have been unaltered since they became distinct in the 60 mm embryos, although it is very difficult to form an idea of their number because the processus anterior of the oral lobe with its vessels covers the plexus more or less completely.

In the following development and growth to the mature stage the pars distalis is enlarged, and the cephalic lobe (the derivative of the oral lobe) extends rostrally and now again covers the main part of the eminentia mediana as is seen from the description of the adult gland (p. 159). A connective tissue layer although rather thin separates the two hypophysial structures and is continuous with the tissue in the recessus transversus.

The development of the vascular system of the neural lobe.

As said above a saccus infundibuli is formed in the 33—40 mm embryos. On the surface of the saccus some capillaries are found, but they do not yet appear in the aboral contact-zone. Vessels in the latter do not develop until in the 60 mm embryo when the mesenchyme has invaded the space between the saccus infundibuli and the anlage of the pars intermedia. With the said completion of the capillary network around the saccus the vascular system of the neural lobe has reached its definite pattern. The plexus of the saccus is from its formation on continuous with the primary plexus. In the following stages the density of capillaries on the saccus increases, but no vessels penetrate the wall of the anlage. The vascular system of the saccus infundibuli is supplied by the posterior rami of the infundibular arteries as in *Lacerta*. The primary communications between the vessels of the saccus and those of the aboral lobe never disappear (cf. p. 161), and they constitute a regular structure in the adult vascular system.

Natrix natrix

Notes on the morphology and vascular system of the hypophysis in the adult.

The main arrangement of the vascular supply of the hypophysis in *Natrix* was described by WINGSTRAND (1951) who also briefly discussed the morphology of the gland. Otherwise only few descriptions were found in the literature although the common grass-snake is often used in anatomical investigations. GENTES (1907) described the morphology of the gland, and POKORNY (1926) used the species in his comparative work.

The main form of the hypophysis in *Natrix* is not ovoid with the long axis

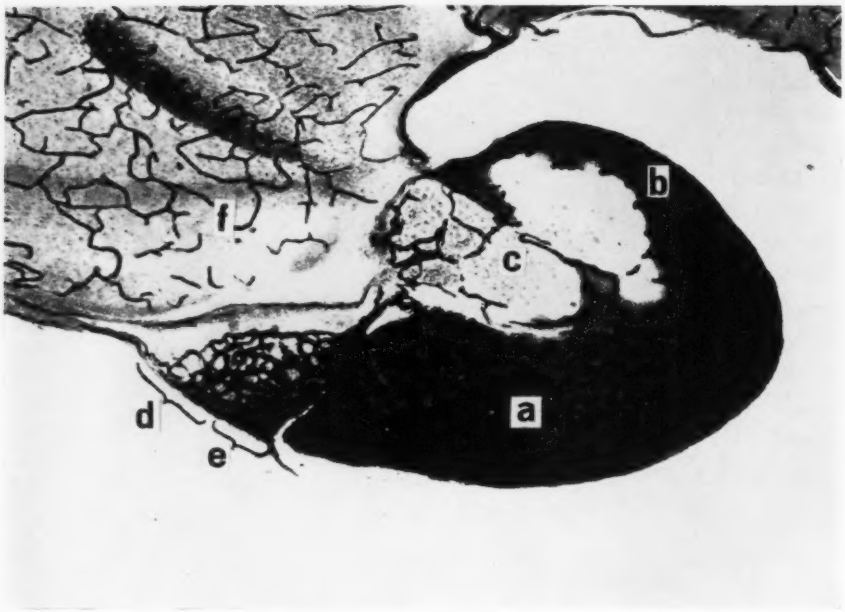

Fig. 14. *Natrix natrix*. Adult. Paramedian section near the midline showing the main organization of the hypophysis. As the hypophysis is asymmetrical the neural lobe is cut tangentially in this section. — a = pars distalis, b = pars intermedia, c = the neural lobe, d = the eminentia mediana with the primary plexus, e = the pars terminalis with the portal vessels, f = tuber cinereum. — Ink injection, celloidin 50 μ, Mallory's phosphostungstic acid haematoxylin, 60 ×.

rostro-caudally orientated as in *Lacerta* and *Anguis*, but it is broader than long (Fig. 16). Further, it is asymmetric as regards its internal organization. Although the composition of the different lobes of the gland is the same as in the reptiles previously described the pars distalis in *Natrix* has turned to one side, and the neural lobe with the pars intermedia to the other. Therefore, the pars distalis and the neural lobe lie side by side as seen in transverse sections of the pituitary. The direction of the said dislocation is not always the same and therefore the neural lobe is found to the left of the pars distalis in one specimen and to the right in another. However, the hypophysis as a whole is almost symmetric, and there are no asymmetric structures in its surroundings. The shallow sella turcica together with the dorsum sellae are perfectly symmetric structures.

The eminentia mediana is a relatively thick structure in sections and its outer wall with the primary plexus is wrinkled and forms very deep and narrow furrows (Fig. 15). Caudally to the eminentia the saccus follows, at first as a thin-walled tube, the infundibular stem, and then thickening to a compact prominent neural lobe, which is composed of numerous secondary lobules densely packed together.

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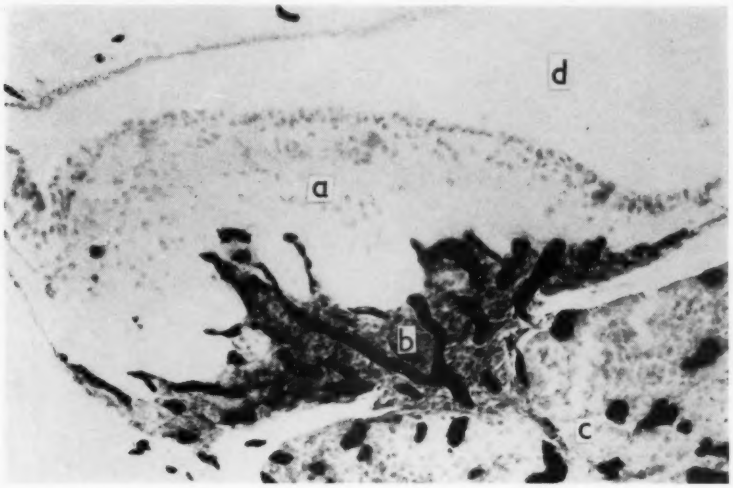

Fig. 15. *Natrix natrix*. Adult. Median section showing the region of the portal vessels. Note the wrinkled surface of the eminentia mediana (a). The capillaries of the primary plexus are seen deep in the furrows. The portal vessels leave the eminentia mediana and run in the connective tissue of the pars terminalis (b) to the pars distalis (c). d = the third ventricle. — Ink injection, paraffin 8 μ, azan, 180 ×.

The third ventricle reaches to about the centre of the neural lobe. In mature specimens the rostral portion of the cephalic lobe covers the whole area of the eminentia mediana. It is, however, well separated from the surface of the latter by a thick layer of dense connective tissue, the pars terminalis, according to SILER's (1936) terminology used in the description of the pituitary in the garter snake, *Thamnophis radix* (Fig. 15). The cephalic and caudal lobes which can be identified histologically, at least to a certain extent (WINGSTRAND 1951, p. 146), constitute the main bulk of the pars distalis. The pars intermedia covers the neural lobe almost completely (Fig. 14), and the tissue of the intermedia is also partly sunk in the furrows of the neural lobe. The pars tuberalis is completely lacking.

The primary plexus is supplied by the infundibular arteries from both sides (Fig. 16). The capillary network is very dense and complicated. Some capillaries of the network are also found, as it would seem, deep in the tissue of the eminentia mediana. They do not penetrate the tissue, however, but are sunk in the very deep furrows of the organ. Thus the contact between the primary plexus and the tissue of the eminentia is very intimate in this species (Fig. 14). The primary plexus is drained by a bundle of portal vessels which converge towards the pars terminalis, penetrates the latter and then diverges and joins the sinus-system of the cephalic lobe (Fig. 15). In older specimens the distance between the rostral end of the cephalic lobe and the eminentia mediana is rather short, and therefore the portal vessels are also short. Their number seems to be greater than in *Lacerta*

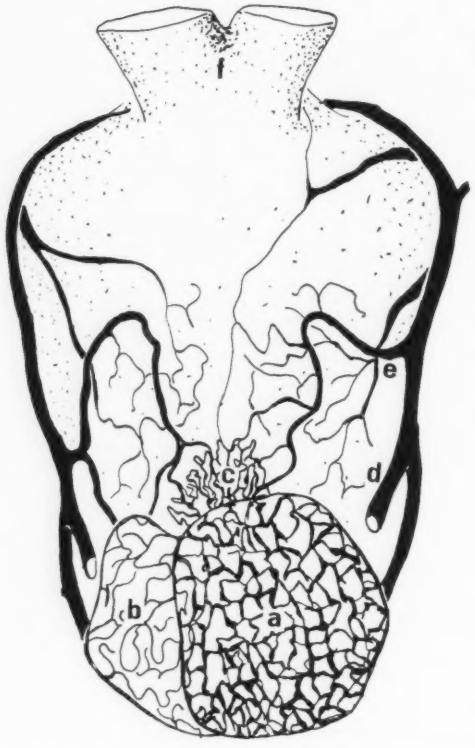

Fig. 16. *Natrix natrix*. Adult. Camera lucida drawing of the vessels of the pituitary region, injected with ink and cleared in benzyl benzoate. Oral view. The vessels of the primary plexus and of the pars distalis have not been drawn in every detail. a = pars distalis, b = pars intermedia, covering the neural lobe, c = primary plexus on the eminentia mediana, d = the internal carotid, e = the infundibular artery, f = chiasma opticum. — The hypophysis is asymmetrical and the neural lobe is dislocated to the right side of this specimen.

and *Anguis*, about seven to eight in the investigated animals. Some capillaries are found on the infundibular stem, and they connect the primary plexus with the vascular system of the neural lobe. This communication is apparently of minute importance in the blood supply of the neural lobe, for the latter is directly supplied by its own arteries, namely the hypophysial arteries running from the internal carotids. A branch from the infundibular artery running to the neural lobe via the infundibular stem was also found in one specimen.

The pars intermedia has a superficial vascular plexus which is very thin compared with those of the eminentia or the pars distalis. The neural lobe is highly vascularized. The capillaries are included in the deep furrows between the numerous lobules of the organ.

In two celloidin-sectioned and ink-injected specimens, small arteries running

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directly from the internal carotids to the pars distalis could be seen. Compare also in this respect the situation at the hatching stage of the species (p. 181).

The development of the hypophysis.

The common grass-snake was often used when the problems of the ontogeny of the pituitary were attacked. Already RATHKE (1839) gives some information on the subject in his classic investigation on the embryology of *Natrix*. Descriptions of some stages were made by SASSE (1886), HOFFMANN (1886), JÄGERSKIÖLD (1890). Also BAUMGARTNER (1916) and HALLER (1924) described the outline of the development. It was, however, necessary to study my own preparations of the hypophysial development to clarify some details, and the result is given below. The description is necessary as a background to the study of the development of the vascular system, for the classification of the embryos was mainly based on the development stage of the pituitary anlage as mentioned above (p. 145).

S t a g e I. Characterization: The hypophysial anlage consists only of the pouch of Rathke, and the latter is in wide communication with the oral cavity (Fig. 17 I).

The lateral extension of the pouch is approximately the same throughout. The epithelium of the anlage is thicker than that of the surrounding oral roof. In very early embryonic stages the rostral wall of the pouch is in intimate contact with the adjacent brain wall. In the earliest stages available for study in the present investigation the mesenchyme has invaded between the epithelium and the brain wall in a transverse zone. Thus the epithelium is loosened from the brain wall in the said zone giving rise to the mesenchyme-filled recessus transversus. Orally and aborally to the recessus transversus the pouch of Rathke is still in contact with the brain in the oral and aboral contact-zones.

S t a g e II. Characterization: The pouch of Rathke is now constricted at its base thus forming the first indication of an epithelial stalk. The lumen of the pouch is still in communication with the oral cavity (Fig. 17 II).

The lumen of the stalk anlage and the epithelial stalk itself has a considerable lateral extension. In a sagittal section, however, the stalk anlage is a slender structure with a narrow lumen. The recessus transversus is widened. In a sagittal section the main part of the pouch gives a U-shaped structure with the U somewhat flattened. This curved shape of the hypophysial anlage was stated already by RATHKE (1839, p. 81). The legs of the U meet the brain surface in the two contact-zones. In the aboral contact-zone the epithelium is still intimately attached to the brain wall. In the oral contact-zone a mesenchyme-filled narrow space separates the brain from the epithelium. It is to be noted that the first proliferation of the epithelium is now visible in the oral contact-zone and in the rostral part of the recessus transversus.

In the present stage the two main lobes of the Rathke's pouch can be discerned,

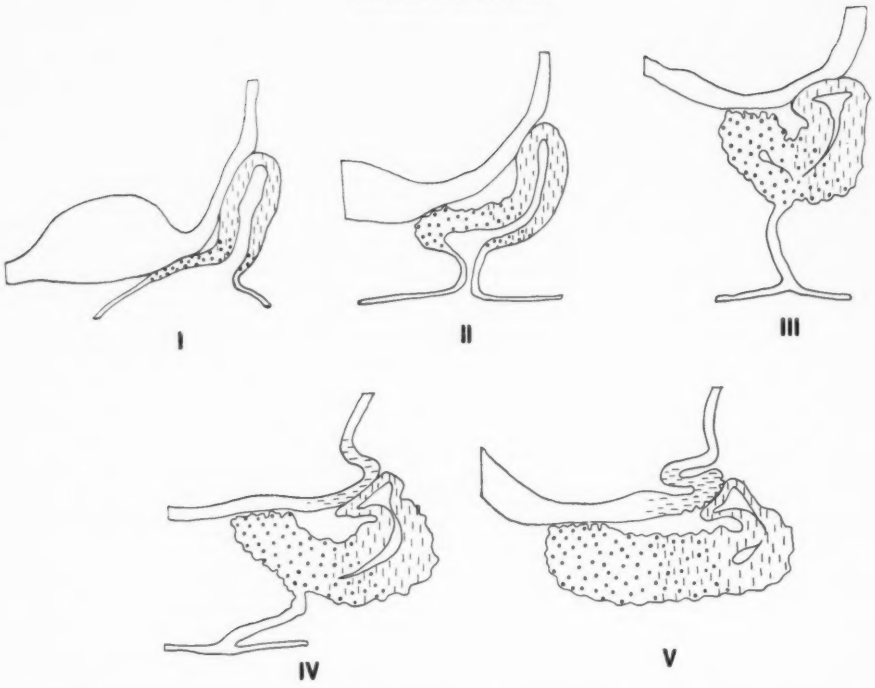

Fig. 17. *Natrix natrix*. Camera lucida drawings of some stages in the development of the hypophysis. Median sections. The stages used in the classification of the embryos (stages I to V) are chosen. For explanations see under Fig. 4.

viz. the oral and aboral lobes. The extension of the two lobes is indicated in the figure. It is however, not possible to fix the exact boundary between the lobes, for only two of the criteria listed on p. 146 and used to separate the lobes of the pouch are applicable to the developing hypophysis in *Natrix* as in other members of *Ophidia*. The lumen of the pouch does not show a clear constriction in *Natrix*. Nor does the pouch form conspicuous lateral lobes. At this stage only a horizontal shallow shelf appears on each side of the oral part of the Rathke's pouch, and perhaps they may be looked upon as rudimentary lateral lobes. They soon disappear completely. The following criteria remain: the oral lobe is indicated by the point of attachment of the epithelial stalk, and the aboral lobe is indicated by the aboral contact-zone. The latter is located to the area where the saccus will develop in later stages. As the development of the hypophysis in *Lacerta* does not show the said aberrations, but results in a U-shaped structure in the same way, WINGSTRAND, after comparisons, could interpret the hypophysial anlage in the snake *Vipera berus*. This interpretation is directly applied to the epithelial anlage in *Natrix*.

To sum up, the oral lobe of Rathke's pouch in *Natrix* consists of the rostral leg

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of the U-shaped anlage. The rostral part of the latter should be designated as the processus anterior containing an anterior diverticle of the lumen. The aboral lobe consists of the caudal leg of the U being the part of Rathke's pouch which in the earliest stages could be distinguished as a pouch-like structure (cf. stage I).

Stage III. Characterization: A compact epithelial stalk is formed (Fig. 17 III).

The epithelium of the oral lobe and the basal parts of the aboral lobe is in intensive proliferation. A mass of cell cords is formed, and the latter grow in all directions, in the oral contact-zone also towards the brain surface. The lumen in the oral lobe is constricted to a narrow tube communicating with that of the aboral lobe. In the latter the lumen has a considerable lateral extension. In the aboral contact-zone the epithelium is still intimately attached to the brain wall.

Stage IV. Characterization: A saccus infundibuli is evaginated, but not yet proliferating. The epithelial stalk is intact (Fig. 17 IV).

The hypophysial anlage is slightly dislocated caudally in relation to the oral roof. The proliferative activity of the anlage embraces most of the aboral lobe except the tip of the lobe which is in contact with the saccus infundibuli. The epithelium of the aboral contact-zone thus constitutes the anlage of the pars intermedia. The lumen of the oral lobe has disappeared. The recessus transversus is a prominent structure. The mesenchyme in the somewhat widened space in the oral contact-zone is condensed and constitutes the anlage of the pars terminalis in the adult.

Stage V. Characterization: The saccus infundibuli is proliferating and the epithelial stalk has disappeared (Fig. 17 V).

The hypophysial anlage is still a symmetric structure. It has a considerable extension in a rostro-caudal direction and appears on the oral aspect as a lengthened structure with parallel lateral borders. The anlage of the pars terminalis is conspicuous, and consequently the brain wall of the oral contact-zone (the prospective eminentia mediana) and the rostral end of the oral lobe (the processus anterior) are separated for a short distance. The aboral lumen still persists, and the tip of the aboral lobe constitutes a relatively thin-walled sac in contact with the proliferating neural lobe. It thus has the characters of the pars intermedia. The remaining aboral lobe and the oral lobe have now developed into large epithelial masses, the caudal and cephalic lobes of the pars distalis. The recessus transversus is represented by a layer of connective tissue.

Stage VI. Characterization: the hatching stage.

The main organization of the hypophysis is the same as in the previous stage, the only difference being that the dislocations resulting in the asymmetric pituitary have occurred. The saccus infundibuli now consists of a neural lobe and an infundibular stem. The neural lobe has turned to one side. The main body of the pars distalis has moved to the other side. Thus it is impossible to give a median section in order to show the main organization of the gland. The rostral end of

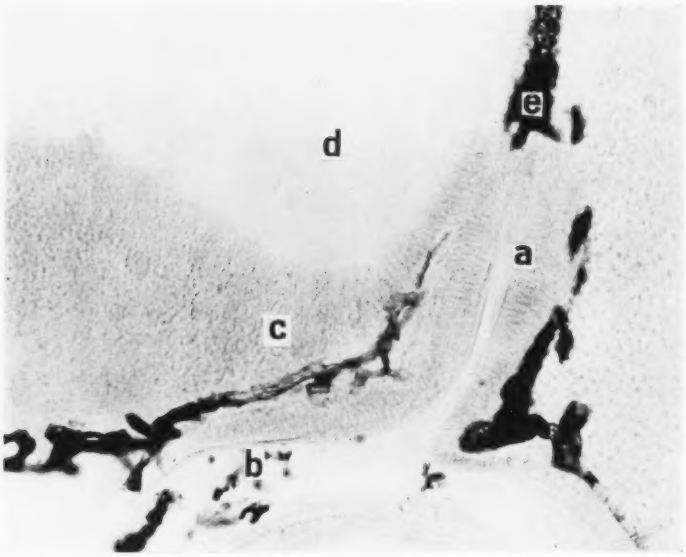

Fig. 18. *Natrix natrix*. Embryo, stage I. Paramedian section of the hypophysial anlage showing the rich capillary plexus surrounding the Rathke's pouch. — a = the aboral lobe, b = the oral cavity with the epithelium of the prospective oral lobe nearby, c = the brain wall, d = the third ventricle, e = vena retrohypophysea. — Ink injection, celloidin 80 μ, 150 ×.

the cephalic lobe is still directed to the eminentia mediana but is separated from the latter by the pars terminalis. The processes resulting in the asymmetric construction of the pituitary have not been studied in detail. It seems as if they do not start until the last days before hatching.

The development of the hypophysial portal system.

S t a g e I. A capillary network covers the surface of Rathke's pouch except the two contact-zones where the epithelium of the pouch and the brain wall are intimately attached to each other. This network is continuous with the capillaries on the surface of the adjacent brain wall (Fig. 18). The internal carotids pass the pouch of Rathke laterally to the aboral lobe, but they do not touch the pouch. The gap between each carotid and the hypophysial anlage is overbridged by an arterial twig which seems to be constantly developed. They are called the hypophysial arteries (arteriae hypophysiae) in the present investigation, and they constitute the main supply to the capillary network of the pouch at this stage (Fig. 19). In a few embryos the left and right hypophysial artery anastomose caudally to the hypophysial anlage, thus giving rise to an intercarotic anastomosis. Such an anastomosis is not found in more advanced stages of development.

The posterior communicating arteries (called aa. cerebrales vertebrales by

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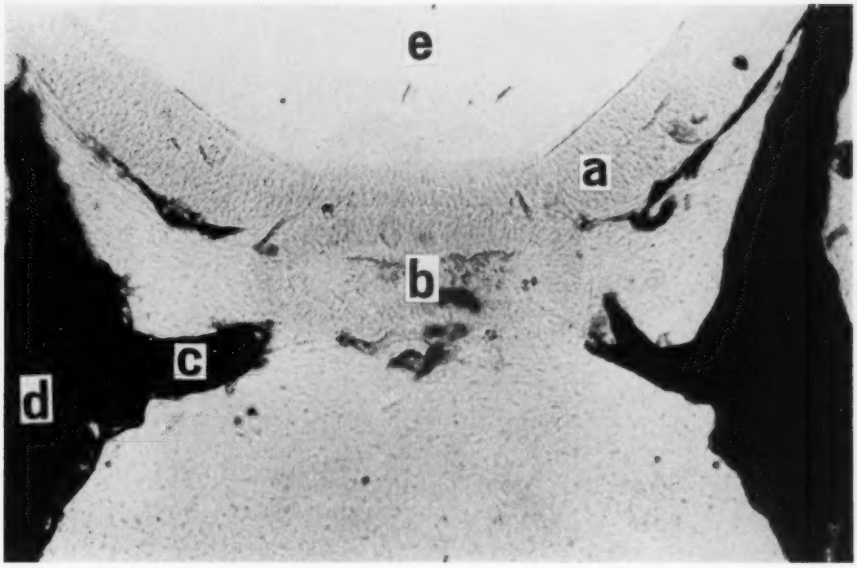

Fig. 19. *Natrix natrix*. Embryo stage I. Horizontal section at the height of the aboral lobe showing the direct arterial supply to the hypophysial anlage. — a = the brain wall, b = the aboral lobe of Rathke's pouch, c = the hypophysial artery, d = the internal carotid, e = the third ventricle. — Ink injection, celloidin 60 μ, 155 ×.

GRODZINSKI (1928) at this stage before their caudal parts have fused to form the unpaired a. basilaris) are branched off just rostrally to the hypophysial arteries. The anterior rami of the internal carotids come into contact with the brain wall, run rostrally, and soon divide into a. ophthalmica and a. cerebralis anterior. Before the said bifurcation they give rise to the infundibular arteries which run orally and are split up supplying the capillary plexus of the hypothalamus. A number of these capillaries runs to and takes part in the blood supply of the capillary plexus surrounding the oral contact-zone, i.e. the part of the Rathke's pouch which later on is constricted of from the oral epithelium and forms the processus anterior of the oral lobe. Thus the vascular system of the hypophysial anlage at this early stage has a double supply by way of the hypophysial arteries on one hand and of the infundibular arteries on the other.

S t a g e II. The density of the capillaries surrounding the hypophysial anlage has increased (Fig. 20). Of the two supplying pairs of arteries the infundibular artery seems to have increased in importance. Though they are branched off from the ramus anterior of the carotids relatively far from the hypophysial anlage as in *Lacerta* and *Anguis* their twigs are easy to follow since they converge orally to the oral contact-zone where they join the surrounding capillary network. Unfortunately, no total preparations of the hypophysial region were made of this

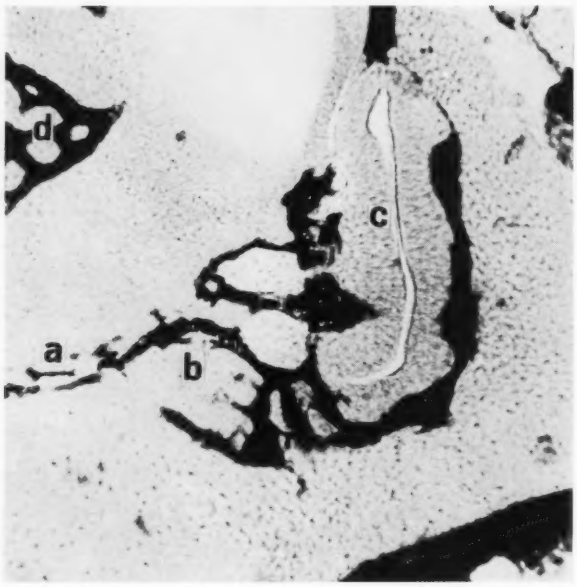

Fig 20. *Natrix natrix*. Embryo, stage II. Paramedian section showing the communication between the vascular plexus of the brain wall and that of the hypophysial anlage. — a = vascular plexus on the brain wall, b = the oral lobe, c = the aboral lobe of Rathke's pouch, d = internal vessels of the tuber cinereum. — Ink injection, celloidin 80 μ, 150 ×.

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species, and therefore it was not possible to get a clear picture of the vascular arrangement in detail as was the case in *Lacerta* and *Anguis*.

Stage III. The plexus of the vessels on Rathke's pouch is now partly included in the proliferating epithelial mass. The cell cords seem to grow into the meshes of the capillary network. The infundibular arteries among others give rise to about three or five orally running thicker vessels on each side of the infundibulum, and these vessels converge to the oral contact-zone (Fig. 22). Probably, these vessels are derived from a number of straight capillaries running on the infundibulum in the previous stages as in *Lacerta* and *Anguis*. The converging vessels unite in the oral contact-zone in a capillary plexus which later differentiates into the primary plexus of the hypophysial portal system. From the congregation of capillaries in the oral contact-zone some capillaries run to the plexus of the oral lobe. These linking capillaries are immediately included in the cell masses of the proliferating lobe, and they cannot be discerned as a bundle of special straight vessels. The two plexuses, that of the prospective eminentia and that of the oral lobe, thus communicate via a set of ordinary plexiform capillaries at this stage. The capillary plexus of the aboral lobe is apparently supplied with blood via the vessels in the oral lobe. Distinct vessels linking the plexuses of the two lobes are seen in the "bottom" of the recessus trans-

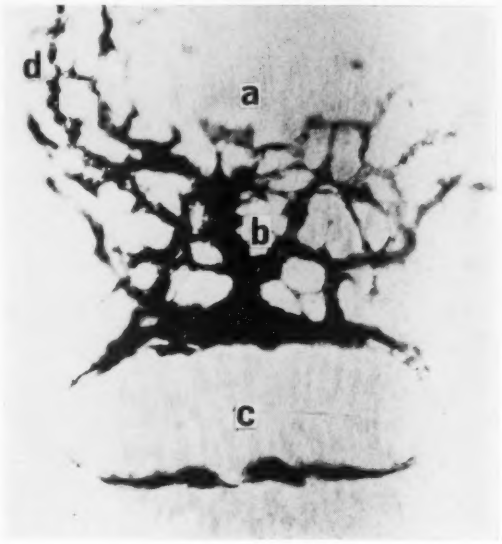

Fig. 21. *Natrix natrix*. Embryo, stage II. Horizontal section, aboral view. The photo shows the vessels which run in the bottom of the recessus transversus along the oral lobe from the oral contact-zone and supply the aboral lobe. — a = the brain wall cut near the oral contact-zone, b = the aboral surface of the oral lobe (being the bottom of the recessus transversus), c = the aboral lobe, d = capillaries on the brain wall running to the oral contact-zone. — Ink injection, celloidin 80 μ, 150 ×.

versus as in the preceding stage (Fig. 21). The hypophysial arteries, as in the preceding stage, run to the plexus of the aboral lobe, but as they now appear to be relatively tiny the infundibular arteries are probably the source of the most important blood supply of the whole hypophysial anlage. The study of the draining pathways from the hypophysial anlage supports this conclusion (p. 216).

Stage IV. The vascular pattern of this stage has not undergone great changes. The vessels in the proliferating lobes are often dilated and should be regarded as sinusoids constituting the anlage of the secondary plexus of the hypophysial portal system of the adult snake. The capillaries which link the plexus of the infundibular wall with those of the adenohypophysial anlage are all located to the oral contact-zone (Fig. 23). It is to be noted that no vessels traverse the recessus transversus on the way from the brain to the hypophysial anlage. Capillaries in the mesenchyme of the recess were only occasionally found. The density of the capillary plexus on the prospective eminentia in the oral contact-zone seems to have increased, but due to the absence of total preparations allowing an oral aspect of the area nothing definite could be said in this respect. The hypophysial arteries were found in all injected specimens of the present stage. They all appeared as relatively thin vessels.

Stage V. The brain wall in the oral contact-zone now has many features of the eminentia mediana in the adult snake. The capillary plexus in that region is now obviously dense. Many capillaries converge towards the contact-zone, and these capillaries are conspicuously thin compared with those in other parts of the brain and of course compared with the sinusoids on the adenohypophysial anlage. It is also remarkable that several capillaries run as straight and unbranched

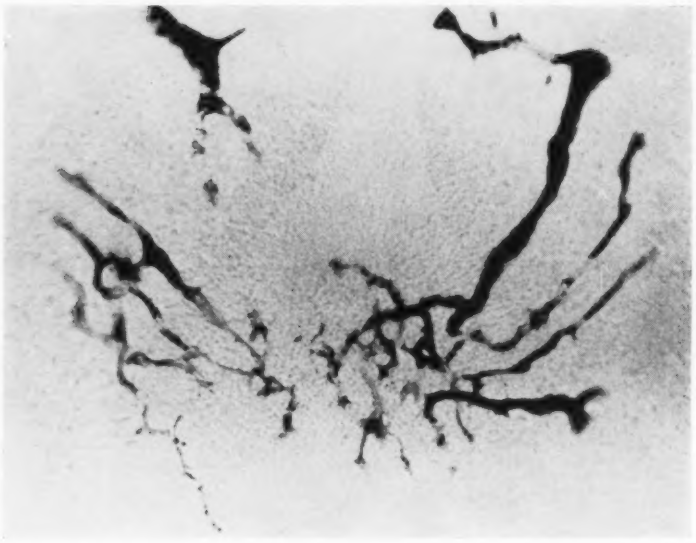

Fig. 22. *Natrix natrix*. Embryo, stage III. Horizontal section comprising the most oral part of the tuber cinereum. It shows the characteristic straight capillaries on the brain wall running to the capillary plexus of the oral contact-zone in the centre of the photo. — Ink injection, celloidin 80 μ, 150 $\times$.

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vessels to the contact-zone starting relatively far from the latter. Thus a horizontal section including this region gives a picture of a bundle of thin papillaries radiating from the capillary congregation in the oral contact-zone. From the said accumulation of vessels in the contact-zone capillaries spread down to the oral lobe and merge into the sinus-system of the latter (Fig. 24). From now on there is a distinct primary plexus beside the secondary one which was undoubtedly distinguishable already in the preceding stages, but these two units of the hypophysial portal system communicate with each other via a broad zone with ordinary capillaries not yet differentiated into typical portal vessels. These cappillaries are embraced by the anlage of the pars terminalis in their course from the surface of the eminentia to the adjacent part of the oral lobe.

S t a g e VI. The last week before the hatching and the period of hatching are characterized by the formation of the portal vessels. Fig. 25 shows a horizontal section with the congregation of capillaries constituting the primary plexus. From there a bundle of radiating capillaries run to the secondary plexus. This bundle is located mainly to the right. The displacement to the right is a result of the moving of the pars distalis which was described above (p. 175) and which results in the asymmetric organization of the pituitary. Fig. 26 shows a horizontal section of another specimen also at the hatching-stage. Here some of the linking capillaries between the primary and secondary plexuses run close together, and they

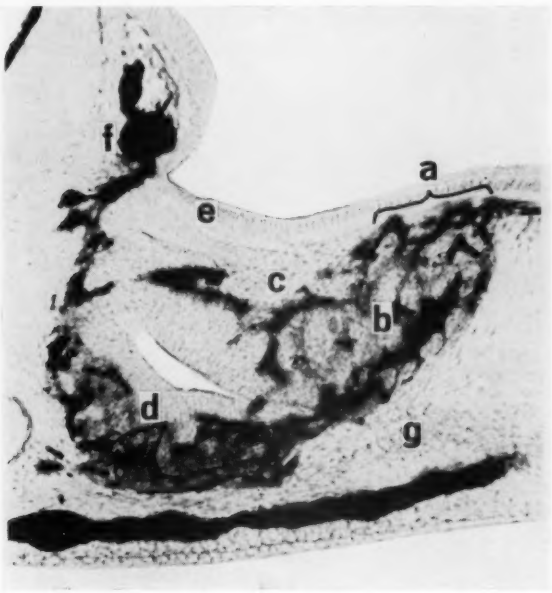

Fig. 23. *Natrix natrix*. Embryo, stage IV. Median section of the hypophysial anlage. The capillaries linking the vascular plexus of the brain wall and that of the hypophysial anlage are located to the oral contact-zone (a). Note that no vessels are found in the recessus transversus (c). A communication between the hypophysial vascular plexus and the vena retrohypophysea is also seen (f). — a = the oral contact-zone, b = the oral lobe, c = recessus transversus, d = the aboral lobe, e = saccus infundibuli, f = vena retrohypophysea, g = the epithelial stalk. — Ink injection, celloidin 80 μ, 145 ×.

are dilated compared with their forerunners. Thus the rise of a bundle of relatively long and unbranched linking vessels, the portal vessels, are at least partly a result of the lateral dislocation of the pars distalis of the hypophysis in *Natrix*. The portal vessels, like their capillary forerunners, are embraced in a conspicuous bulk of connective tissue, the pars terminalis.

The very complicated accumulation of vessels in the photo (Fig. 25) not only shows the vessels of the primary plexus, but also the capillaries leaving the latter on entering the pars terminalis. The surface of the eminentia mediana shows shallow furrows at this stage, but the deep and complicated arrangement of furrows apparently does not appear until in the period after hatching. The inclusion of the capillaries in deep furrows is a process which has not been studied since no stages between the hatching stage and the mature stage were available.

The hypophysial arteries running from the internal carotids to the caudal lobe still exist. They are however small, and it must be concluded from a comparison with the portal vessels that the hypophysial arteries are of less importance in the supply of the pars distalis. An "arteria hypophysealis" from the posterior com-

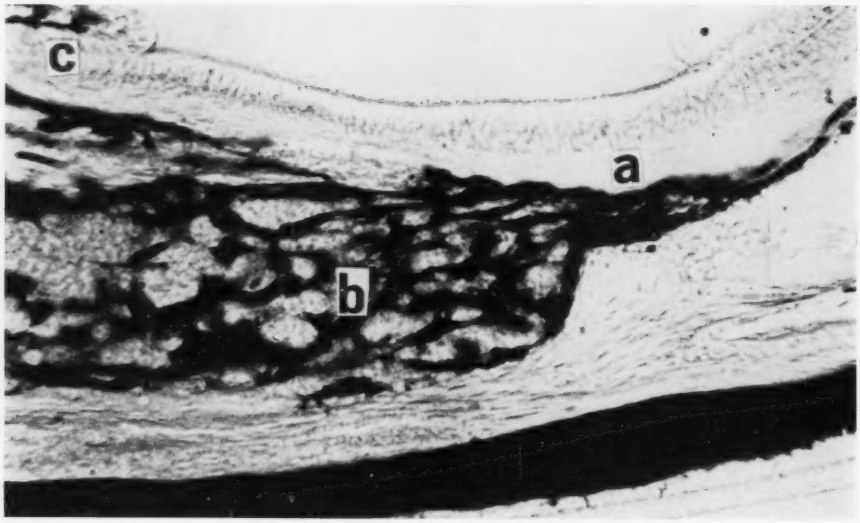

Fig. 24. *Natrix natrix*. Embryo, stage V. Median section of the anterior part of the hypophysial anlage showing the linking capillaries between the primary plexus on the eminentia mediana (a) and the secondary plexus of the oral lobe (b) in the stage before the portal vessels are formed. c = saccus infundibuli. — Ink injection, celloidin 80 μ, 230 ×.

municating artery to the hypophysis was described by GRODZINSKI (1928, p. 24), but such a vessel was never found in the present investigation.

The development of the vascular system of the neural lobe.

Stages I and II. The anlage of the neural lobe, the saccus infundibuli, is not yet formed. The corresponding part of the brain wall is that of the aboral contact-zone. The aboral lobe is here closely attached to the brain wall, leaving no space in between.

Stage III. A very thin layer of the mesenchyme has invaded the space between the aboral lobe and the brain wall. No capillaries are formed except in the most peripheral parts of the contact-zone.

Stage IV. The newly established saccus infundibuli is surrounded by capillaries throughout. They are also found in the aboral contact-zone between the anlage of the pars intermedia and the saccus infundibuli. The capillaries are continuous with those of the adjacent parts of the brain and of the aboral lobe.

Stage V. The capillary plexus is now included among the proliferating lobules of the saccus infundibuli. Thus the vascular system of the neural lobe is formed. A special vascular supply has not been found. The whole hypophysial anlage seems to be supplied with blood via the infundibular arteries although the hypophysial arteries take a minor part in it. The communications of the latter

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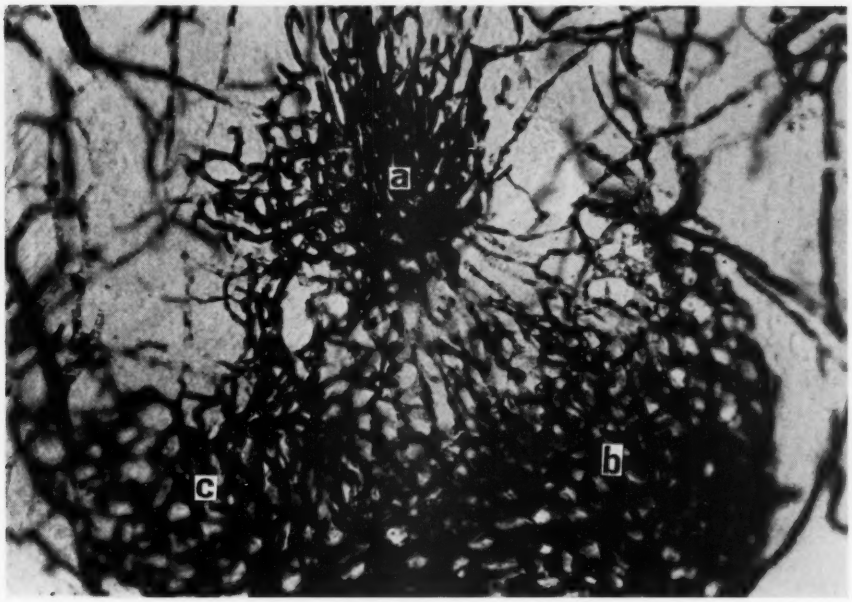

Fig. 25. *Natrix natrix*. Hatching stage. Horizontal section showing a number of outstretched thin capillaries, the forerunners of the portal vessels, between the primary plexus (a) and the secondary plexus of the oral lobe (b). c = the vascular plexus of the neural lobe. The oral lobe has turned to the right and the neural lobe to the left when the linking capillaries were stretched and therefore the organization of the pituitary becomes asymmetrical. — Ink injection, celloidin 100 μ, 150 ×.

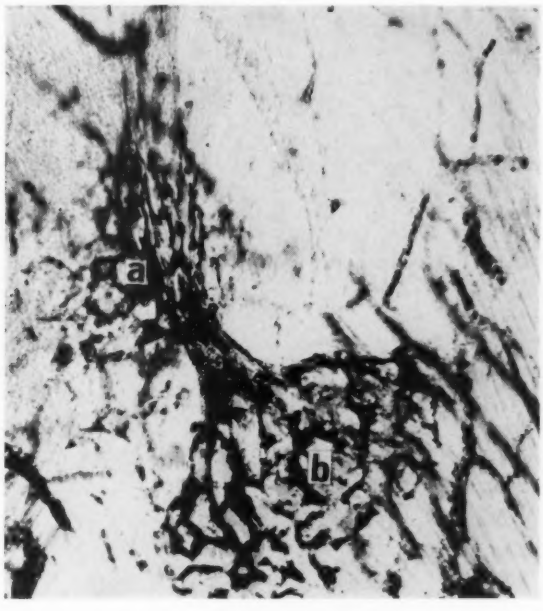

Fig. 26. *Natrix natrix*. Hatching stage. Horizontal section showing the stage which succeeds that of Fig. 25. A bundle of typical portal vessels is seen connecting the primary plexus (a) with the secondary plexus (b). All portal vessels are not included in this section. — Ink injection, celloidin 100 μ, 150 ×.

with the vessels of the neural lobe have not been possible to state. No doubt they terminate in the hypophysial anlage near the saccus infundibuli.

Stage VI. The vascular system of the neural lobe is now relatively well separated from that of the adenohypophysis. The processes forwarding this separation will be discussed later on (p. 208). The lobe is supplied by the hypophysial arteries of both sides. At this stage the neural lobe is bent to one side in the asymmetric hypophysis. The hypophysial artery from the internal carotid of the opposite side is always found running dorsally to the adenohypophysis and terminating in the vascular system of the neural lobe. Small branches from the hypophysial artery to the sinus-system of the pars distalis are also found. The main function of the hypophysial artery at this stage seems to be to supply the neural lobe with blood. An artery from one of the infundibular arteries running to the neural lobe was also found in this stage. Capillary connections between the vascular plexus of the neural lobe and that of the pars intermedia still remain.

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COMPARISONS AND DISCUSSIONS

Though the embryological investigations dealing with the development of the pituitary vessels in the *Amniota* are not very numerous they now comprise all the amniotic classes. Therefore, it is possible to start a discussion on the different classes from a comparative point of view. Such comparisons were carried out to a minor extent in an earlier paper (Enemar 1957), and the author now attempts to bring these comparisons a step forward including the new experience obtained from the reptilian material. However, the conclusions in the following comparative deductions must partly be regarded as preliminary, but they may be of some value to future authors when a larger material including other species have accumulated. It must also be borne in mind that the complete lack of information of the development of the hypophysial vascular system in *Anamnia* may cause misinterpretations when discussing the amniotic conditions.

The comparative discussions are focussed on the developing structures, but sometimes of course the conditions in the adult are referred to. It should be pointed out here, that the literature on the adult pituitary vascular systems are not totally reviewed in this paper. (Cf. e.g. Green 1951, Benoit and Assenmacher 1955.)

The blood supply of the hypophysis in the adult reptiles.

As regards the vascular arrangement of the hypophysis comparatively few reptiles have hitherto been investigated. The presence of a hypophysial portal system has however been stated in all cases, and there is no reason to doubt that this arrangement of vessels is universal in the reptilian class.

The hypophysial vessels in *Chelonia* have been studied on several occasions.

The development of the hypophysial vascular system

GREEN (1951) described portal vessels in *Chrysemys picta* and the vascularity of the different lobes of the pituitary in the same species was investigated by TAYLOR (1952). DIEPEN (1952, 1955) gives some information of the hypophysial vascular system in *Testudo graeca* with special reference to the primary plexus.

The only description of the hypophysial vascular system in *Crocodilia* is that given by GREEN (1951) dealing with *Alligator mississippiensis*. The features of this species were found to agree with those of the chelonians investigated by the same author.

In the *Lacertilia* GREEN (op. cit.) studied the hypophysial portal system in *Anolis carolinensis*. DIEPEN (1955) comments on the vessels between the eminentia mediana and the pars distalis i *Anguis*. The descriptions in the present investigation confirmed the presence of a typical hypophysial portal system in *Lacerta agilis* and *Anguis fragilis*. The portal vessels were also stated to constitute the only blood supply to the part distalis, since a direct arterial supply to the part distalis was not found in the adult specimens or in the embryos.

Also a few species of *Ophidia* were studied. GREEN (op. cit.) found hypophysial portal vessels in the garter snake *Thamnophis*, and WINGSTRAND (1951) described the vascular system of the hypophysis in *Natrix natrix* and *Vipera berus*. He also found the same arrangement of the portal system in *Coronella*. DIEPEN (1955) showed the portal vessels in the pars terminalis of *Vipera berus*.

A comparison of the hypophysial vascular system of the three reptiles in the present investigation clearly shows that the system of *Natrix* is the most advanced. The snake has a more complicated primary plexus than the lizards which means an increased vascular contact with the eminentia mediana. Further, the vascularization of the neural lobe is richer in *Natrix* where the vessels are included in the furrows of the lobe and seem to constitute an internal vascular plexus. The blood circulation of the neural lobe is also relatively well separated from the portal circulation in *Natrix*. In this species special arteries supplying the lobe are developed, and there are only few capillary connections on the infundibular stem between the primary plexus and the vascular system of the neural lobe. *Lacerta* and *Anguis* have a simple primary plexus which is a flat and superficial network on the eminentia. The vascular plexus of the whole neurohypophysis is more uniform in these species, especially in *Anguis*. In the last mentioned species with its primitive sac-like neural lobe the vascular plexus of the latter is continuous with the primary plexus, a feature which might be looked upon as primitive. Hypophysial arteries running to the neural lobe are not found in the two lizards. The latter is supplied by branches from the infundibular arteries beside the blood flow via the capillaries from the primary plexus.

On the direct arterial supply to the pars distalis.

The hypophysial portal vessels deliver the whole quantity of the blood to the pars distalis in *Lacerta* and *Anguis* and the main quantity also in *Natrix*. This

agrees with the statements as to the blood supply to the pars distalis also in other amniotes. However, the hypophysial arteries when present no doubt supply the sinus-system of the pars distalis. Such direct arterial supply to the lobe has been demonstrated for several amniotic species, but there has been some discussion in the literature as to the existence and significance of this direct supply. The hypophysial arteries probably have sometimes been overlooked, owing to the fact that the main interest has been paid to the hypophysial portal system with its important functional properties. It should however be remembered that the existence of functioning hypophysial arteries does not exclude the activity of the hypophysial portal vessels. The two types of supplying vessels can work side by side.

As shown by WINGSTRAND (1951) a direct arterial supply to the pars distalis is regularly found in the adult *Vipera*. This is the case also for *Natrix*, and the said supply is laid down very early in the embryonic life of the species as appears from the present investigation. Judging from the size of these arterial branches they seem to constitute the main supply to the capillary plexus of the hypophysial anlage in the earliest stages of the latter. However, the capillaries in the most anterior part of the hypophysial anlage, in the region of the oral contact-zone, show clear connections with the infundibular arteries, and consequently receive their blood from these arteries. The infundibular arteries gradually increase in importance as tributaries to the hypophysial vascular system. The hypophysial arteries are retained as supplying branches also in the adult snake, but their internal diameter is very small, and their tribute to the pars distalis must be negligible compared with the blood stream reaching the lobe via the portal vessels.

In *Lacerta* and *Anguis* no hypophysial arteries were found in the adult specimen, and as regards the embryos arterial branches to the vascular plexus of the hypophysial anlage never appeared during the development. The only indication was a very thin intercarotic anastomosis in a single embryo of *Lacerta* and also in a very early embryo of *Anguis*, but it was not possible to detect any communication between this vessel and the capillaries of the hypophysial anlage.

WINGSTRAND (1951) investigated the supplying path-ways to the pituitary in a number of bird species, but did not find any other afferent vessels than the portal ones running to the pars distalis. ASSENMACHER (1951) working with the domestic duck described directly supplying vessels from the infundibular arteries (the superior hypophysial arteries) and the (inferior) hypophysial arteries, and they may exist side by side. The occurrence ond the course of the inferior hypophysial arteries are however inconstant and variable. This is the case also in the domestic fowl according to GRIGNON (1956). The cited authors do not report any arterial supply in the early hypophysial anlage corresponding to the hypophysial arteries in *Natrix*. Assenmacher did not detect arteries to the anlage until the latter had begun to proliferate (after 13 days of incubation). Grignon found an arterial branch from the region of the intercarotic anastomosis penetrating the posterior wall of the hypophysial anlage in an embryo incubated seven days.

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The development of the hypophysial vascular system

In mammals a direct arterial supply to the pars distalis is stated in some instances (*Lepus,* HARRIS 1947). XUEREB, PRICHARD and DANIEL (1954 a, b) showed that the artery running to the pars distalis of the pituitary in man does not empty into the sinus-system of the lobe, but supplies the capillary bed in the eminential tissue of the lower infundibular stem. Such a special supply of the lower infundibular stem was detected also in *Ovis* by DANIEL and PRICHARD (1957), though the vessel in question did not traverse the pars distalis on its way. These observations are of great importance when interpreting the significance of the arteries running to the pars distalis.

LANDSMEER (1947, 1951) and DANIEL & PRICHARD (1956) found no arteries running to the pars distalis in the rat, and such vessels were not found in *Clethrionomys glareolus* (ENEMAR 1957). As regards the last mentioned rodent ten individuals were injected with india ink from the heart after sectioning the infundibular arteries near their bases on the carotids (ENEMAR unpublished). None of these preparations showed any artery emptying into or traversing the pars distalis. An arterial supply to the pars distalis in *Ovis* is also missing according to DANIEL & PRICHARD's careful investigation (1957).

As regards the early developmental stages of the mammalian pituitary vessels only few comments are made on the existence of a direct arterial supply to the hypophysial anlage. In *Clethrionomys* an arterial branch was detected in early stages in a very restricted number of embryos (ENEMAR op. cit.). GLYDON (1957) considers the vessels following the epithelial stalk to be supplying pathways to the oral part of the hypophysial anlage in the laboratory rat. The blood stream in these vessels is no doubt the reserve, and the vessels will be dealt with later on (p. 229). BOERNER-PATZELT (1954) found arterial branches growing from the carotids into the anlage of the pars distalis in the embryo of *Cavia* and *Homo,* but these observations must, at least partly, have been misinterpreted as Boerner-Patzelt studied the development of the vessels in the hypophysial anlage without mentioning the presence of the hypophysial portal vessels or their forerunners.

The hypophysial arteries take their origin from various sources, e.g. the intercarotic anastomosis in birds and the internal carotid in *Natrix*. They are obviously not homologous in all cases and therefore it is difficult to discuss their phyletic significance. In addition, the number of investigated species is too small. The condition found in *Natrix,* i.e. large hypophysial arteries supplying the early adenohypophysial anlage, has not yet been described from other amniotic species.

The general course of the development of the hypophysial vascular system in the Amniota.

The obvious similarity in the arrangement of the hypophysial vascular system in the different classes of *Amniota* was pointed out by GREEN (1951) and WINGSTRAND (1951), and they also considered that this similarity supported the view

that the hypophysial portal system has functional properties of great significance. If so one would expect the general course of development of the system to be practically the same. In fact, this is the case and on the basis of the author's own investigations and the literature referred to below a short characterization of the development can be given.

As regards the mammals detailed descriptions of the development of the hypophysial vascular system covering the whole period of the intra-uterine life did not appear until 1957. This year investigations on some rodents species were published by GLYDON (the laboratory rat) and ENEMAR (the bank-vole, *Clethrionomys glareolus*, and the yellow-necked mouse, *Apodemus flavicollis*). Recently MIRAGLIA (1958) has reinvestigated the development of the hypophysial vessels in the laboratory rat. Already in 1937 WISLOCKI published a paper on the early development of the hypophysial portal system in man, and, apart from his interpretations of the development of the portal vessels, Wislocki's paper is the first correct description on this subject. It was however based on a very small number of embryos and comprised only a part, though a very important part, of the period of development. NIEMINEVA published in 1950 (a, b) some observations on the vascularity of the pituitary and the growth of the portal vessels in the human foetus from the middle of the gestation period and onwards. Also BOERNER-PATZELT (1954) has studied the development of the vascular plexus of the hypophysis in the human embryo. Her investigation was restricted to the study of the relations between the proliferating epithelium and the surrounding capillary bed. Boerner-Patzelt also dealt with the guinea-pig, *Cavia cobaya*. Already in 1933 'ESPINASSE published a paper on the development of the hypophysial portal system in *Homo*. 'Espinasse however had an obviously wrong idea of the circulation of the blood in the hypophysial vessels and his statements have been criticized by later authors. It is not possible to include his results in the general survey below. The literature also contains several short comments on the development of different features in the hypophysial vascular system. BECKER (1955) mentioned some data in the development of the primary plexus in the laboratory mouse, and HARRIS (1947, 1955) discussed the rise of the portal vessels.

The embryology of the hypophysial vascular system in birds is wellknown due to the careful investigations made by WINGSTRAND (1951) who studied *Gallus gallus*, *Larus ridibundus*, and *Riparia riparia*, and by ASSENMACHER (1951, 1952) who examined *Anas* (the domestic duck) and, finally, by GRIGNON (1954, 1956) who also studied *Gallus* (the fowl Rhode-Island). All these authors agree as to the main conclusions.

As previously pointed out (p. 143) no information as to the development of the hypophysial vascular system in *Reptilia* is found in the literature, and therefore all references to the reptiles are based entirely on the descriptions given in the present paper.

Regarding the amniotic species hitherto investigated the following summarizing

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characterization is common for the development of the hypophysial vascular system.

1. The early hypophysial anlage consisting of the wide-open pouch of Rathke is surrounded by a plexus of capillaries which is continuous with the capillary plexus on the adjacent brain surface. Thus the vascular system of the epithelial part of the hypophysis, the adenohypophysial anlage, is from the very beginning in communication with that of the brain wall. This is of course true also for the capillaries on the saccus infundibuli when the latter appears. The capillaries of the epithelial and neural parts of the hypophysial anlage constitute a uniform network. The capillaries in the region of the hypophysial anlage are supplied by infundibular arteries. This supplying path is constantly developed although it may function together with more posteriorly located supplying vessels from the carotids.

2. The secondary plexus of the hypophysial portal system is established when the capillaries surrounding Rathke's pouch are included among the proliferating buds or cell cords of the growing pouch. The vascular system in the anlage of the pars distalis always retains its original communication with the capillaries on the adjacent brain surface, the floor of the tuber cinereum.

3. The capillary plexus on the just mentioned part of the infundibular wall being the anlage of the eminentia mediana becomes denser compared with the capillary network on the adjacent brain wall, and it can thereafter be recognized as the primary plexus of the hypophysial portal system. The original capillary connections between the area of the primary plexus and the vascular system of the adenohypophysial anlage remain unchanged.

4. The saccus infundibuli proliferates, and its vascular system becomes more efficient as it grows into a dense network. In the higher forms the latter is included in the neural lobe tissue. It is often sunk into furrows of the lobe, but it also remains as a superficial network in other species. Special arterial branches from the infundibular arteries or from more posteriorly located arteries or from both are developed to supply the neural lobe.

5. The secondary plexus withdraws from the primary plexus. The original capillaries connecting the two plexuses are transformed into the portal vessels, i.e. they become relatively long and unbranched and their internal diameter becomes greater.

6. The vascular system of the neural lobe, though originally in intimate communication with that of the adenohypophysial anlage, becomes more or less completely separated from the hypophysial portal circulation.

In spite of the agreement in the general course of development of the hypophysial vascular system in the three amniotic classes a closer comparison unveils certain dissimilarities, which may be due to differences in the anatomy of the pituitary or of the pituitary region. These deviations will be dealt with in the following.

The anlage of the hypophysial portal system and the pituitary homologies of Woerdeman and Wingstrand.

The problem first suggesting itself when comparing the *Sauropsida* with the *Mammalia* is the homologies of the pituitary and its developmental stages. To what extent does the study of the early development of the hypophysial portal system support or contradict the conclusions drawn by WOERDEMAN (1915) and by WINGSTRAND (1951) in this respect (cf. p. 196).? This is a problem of great theoretical interest, and will therefore be thoroughly treated on the basis of the developing hypophysial vessels. Thereby, the behaviour of the epithelium of Rathke's pouch in the region of the oral contact-zone and the capillaries linking the vascular plexuses of the infundibulum and the pouch will be compared in detail in the three classes. This discussion is found in the first section under the present heading and illustrated in Fig. 27. In the second section some epithelial structures in the hypophysial anlage of the *Mammalia* are interpreted since the said linking capillaries play a rôle also there.

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A. The relation between the epithelium of the oral contact-zone and the capillaries linking the anlages of the primary and secondary plexuses (Fig. 27).

The anterior wall of the prospective Rathke's pouch originally, from the formation of the buccal cavity, adheres to the wall of the infundibular anlage. This is pointed out by several authors in opposition to the old view that the pouch evaginated from the oral epithelium when the latter had already been separated from the neural tube. This primary contact between the brain wall and Rathke's pouch is generally valid for the *Amniota*. During the development, however, this contact almost completely disappears, but persists in comparatively old stages in the region of the tip of the pouch, the aboral contact-zone, where the pouch develops into the pars intermedia in the adult in several amniotic groups. This process is not exactly identical in the three amniotic classes as regards the oral contact-zone, and a survey is given below on this subject with special attention paid to the course of the capillaries connecting the brain with the hypophysial anlage.

As regards the reptiles the development of the epithelial pouch of *Lacerta agilis*, *Anguis fragilis*, and *Natrix natrix* has been described earlier (p. 151, 161 and 173). The epithelium of the pouch remains attached to the brain wall in two regions, the oral and aboral contact-zones. Between these contact-zones the epithelium is detached from the brain, in that way forming a conspicuous mesenchyme-filled recess, the recessus transversus, which appears as a groove between the anterior process of the oral lobe and the aboral lobe. This recess is not used as a path for the capillaries linking the plexus of the brain wall and that of the adenohypophysial anlage. The linking capillaries only run in contact with the processus anterior of the oral lobe, and it should be noted that this part of Rathke's pouch from the

beginning is in contact with that part of the neurohypophysial anlage where the primary plexus of the hypophysial portal system develops later on (Fig. 27 A and B). During the greater part of the embryonic life the tip of the anterior process is separated from the brain by a very thin layer of mesenchyme which is consequently traversed by the linking capillaries. This mesenchyme with its capillaries corresponds to the pial hypophysial mesenchyme of WISLOCKI (1937) (cf. p. 195).

The literature on the development of the hypophysis in *Reptilia* shows that the process of development as stated for the three species in the present investigation is generally valid for *Lacertilia* and *Ophidia*. As regards *Lacertilia,* the early formation of a processus anterior and a recessus transversus is present also in *Lacerta muralis* (WOERDEMAN 1915, DE BEER 1926), *Gongylus ocellatus* (BRUNI 1914, ORRÙ 1900), and *Platydactylus* (MELCHERS 1899). In addition, it is possible to trace the existence of a transverse recess also in the descriptions of the adult pituitary glands, e.g. in *Xantusia vigilis* (MILLER 1948) and *Anolis carolinensis* (PORIS & CHARIPPER 1938). Concerning *Ophidia* it is difficult to get information from other species than *Natrix natrix*. The same mode of development is true for *Vipera berus* (WINGSTRAND 1951), and *Eutaenia sirtalis* (BAUMGARTNER 1916). However, nothing is to be found about the developing blood vessels of the hypophysis in the said species, but it is very probable that the condition is the same as in three species dealt with in the present investigation, namely that the early formed anterior process of the oral lobe receives the capillaries linking the prospective primary and secondary plexuses of the hypophysial anlage.

The development of the hypophysis in *Sphenodon punctatus* has been studied by WYETH and ROW (1923). Sagittal sections of two early stages show a recessus transversus exactly as in *Lacertilia* and *Ophidia,* but the illustrations of the succeeding stages show that it is not equally developed as in the said groups. The sagittal section of a later developmental stage (embryo S) shows a connective tissue layer probably corresponding to the recessus transversus. A paramedian section of the same stage, reproduced by DE BEER (1926) shows the same structure.

The development of the hypophysis in *Crocodilia* was described by REESE (1910) and by BAUMGARTNER (1916), both dealing with the American alligator. In this species the oral part of the rostral wall of Rathke's pouch does not remain attached to the brain wall in the early embryonic stages, and thus no conspicuous processus anterior and recessus transversus are formed. However, the proliferation of the oral lobe of the pouch results in the anlage of the cephalic lobe which secondarily reestablishes the close contact with the brain wall, probably in the region of the prospective primary plexus and the eminentia mediana. The general course of the development of the oral lobe seems to resemble that in *Aves*.

The early embryology of the hypophysis in *Chelonia* has been studied in a surprisingly large number of species. E.g. ZELENY (1901) investigated *Aspidonectes spinifer* and *Chelydra serpentina,* WOERDEMAN (1915) *Chrysemus picta,*

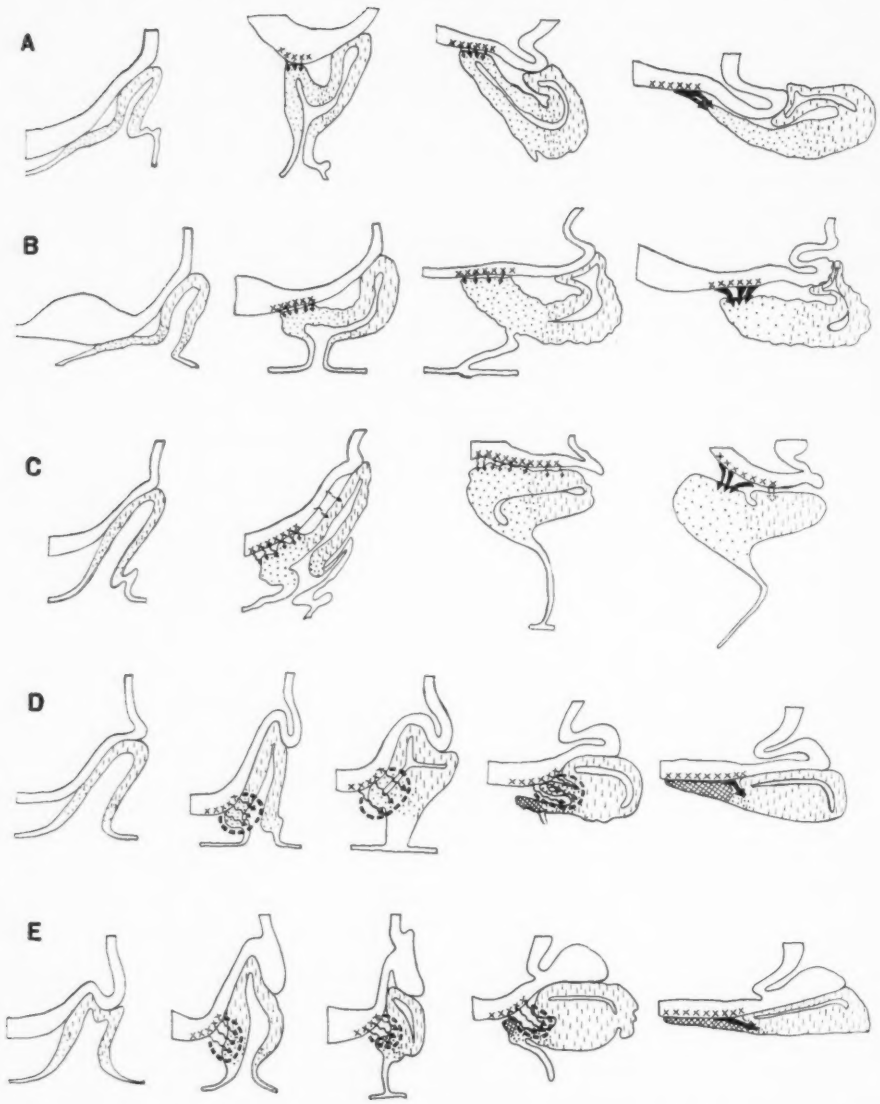

Fig. 27. Diagrams showing the relation of Rathke's pouch to the developing primary plexus and portal vessels in specimens of the three amniotic classes (A and B reptiles, C bird, D and E mammals). Each sequence starts with the stage when the anterior wall of Rathke's pouch has loosened from the brain wall in the way which is characteristic of the species, and it ends with the stage at which distinct portal vessels first appear. The vascular link between the brain and the hypophysis is located to the oral contact-zone (reptiles, birds) or to Atwell's recess (mammals). Dots = oral lobe, short vertical lines = aboral lobe, crossed lines = lateral lobe, thin waving arrows = linking capillaries connecting the primary plexus or its forerunner with the vascular system of Rathke's pouch, heavy arrows = portal vessels, crosses = the primary plexus or its anlage, curved broken line in D och E denotes

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and BAUMGARTNER (1916) *Chrysemys marginata* and *Aromochelys odorata*. SPRANKEL (1956) studied *Testudo graeca* and *Emys orbicularis*, but only the latter species comprised the stages of interest in the present connection. In all species a processus anterior is formed from the oral part of the rostral wall of Rathke's pouch when the base of the pouch is constricted. Sprankel also interpreted the said structure as the processus anterior of the oral lobe in his excellent study. When the anterior wall of Rathke's pouch is loosened from the brain wall it is not dislocated to the same extent as in *Lacertilia* and *Ophidia*, and therefore a deep recessus transversus is not formed. Though the development of the vascular system has not been thoroughly studied, it is obvious that the linking capillaries are located to the region of the oral contact-zone, running from the brain mainly to that part of the adenohypophysial anlage which is formed by the anterior process. This is confirmed by SPRANKEL's (1956, p. 633) statement: "Eine durchlaufende gefässführende Bindegewebslamelle findet sich jetzt in ganzer Ausdehnung des Kontaktes zwischen Adenohypophyse und Boden des dritten Ventrikels; nur im Bereich des Saccus infundibuli bleibt sie gefässarm." This description refers to stages at which the pouch was intensively proliferating and with the epithelial stalk existing. It is evident that Sprankel described the pial-hypophysial mesenchyme of WISLOCKI (1937). It is thus represented by a comparatively thin layer of mesenchyme in *Chelonia*.

In *Aves*, according to WINGSTRAND's monograph (1951), the epithelium of Rathke's pouch does not remain attached to the brain in the oral portion of the pouch, but the epithelial layer is detached (Fig. 27 C). The primary contact between the pouch and the brain in the oral contact-zone is thus lost very early in the development. The oral lobe proliferates as does the aboral one, the former giving rise to the material of the cephalic lobe in the adult. The anlage of this lobe grows on to the brain wall and finally covers the region where the primary plexus of the hypophysial portal system will develop. The oral contact-zone is thus reestablished in spite of the fact that the primary contact by means of an anterior process has been abandoned. An anterior diverticulum is nevertheless formed in some species, as in *Larus ridibundus* (WINGSTRAND). As may be expected no recessus transversus is developed although small traces of it may be

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the localization of Atwell's recess. A = *Lacerta agilis*. The diagrams are based on the results of the present investigation. B = *Natrix natrix*. According to the present investigation. The direction of the portal vessels is not oral as in the figure. They are drawn out caudo-laterally, not possible to indicate in the median section. C = Bird. As to the embryology of Rathke's pouch that of *Gallus gallus* according to WINGSTRAND (1951) is chosen. The arrangement of vessels is based on the interpretations of WINGSTRAND, ASSENMACHER (1952), and GRIGNON (1956). Dotted arrows indicate the posterior portal vessels and their forerunners. D = *Clethrionomys glareolus*. According to ENEMAR (1957). Note the formation and regressive development of the oral lobe (processus anterior), and the length of the linking capillaries in Atwell's recess. E = Laboratory rat. The embryology is based on SCHWIND (1928) and the vascular arrangement on GLYDON (1957).

found in the region of the constriction of the lumen of Rathke's pouch between the oral and aboral lobes, as seen in Wingstrand's serial drawings of the pituitary development in *Larus, Riparia,* and *Gallus.*

The name pial hypophysial mesenchyme (Wislocki 1937) was used by Assenmacher (1952) and was applied to the relatively thin layer of vascularized mesenchyme separating the proliferating epithelium of Rathke's pouch from the brain wall in *Anas,* also by Grignon (1956) who described the corresponding structure in *Gallus.* According to Grignon the pial hypophysial mesenchyme is first formed between the brain wall and the "segment antérieur" of Rathke's pouch, and the said segment of the pouch early begins to proliferate and include the pial hypophysial mesenchyme with its vessels among the cell buds of the pouch. The "segment antérieur" exactly corresponds to the oral lobe of Wingstrand, and therefore the established contact between the said segment and the brain is the oral contact-zone and the pial hypophysial capillaries (les veinules pie-mériennes) are the linking capillaries between the prospective primary and secondary plexuses. It is evident from Assenmacher's illustrations and Grignon's description that the linking vessels are concentrated to the oral contact-zone and that they are therefore taken up mainly by the oral lobe. These vessels then take part in the formation of the porto-tuberal tract (the bundle of portal vessels embraced by the tissue of the pars tuberalis). The latter always bridges the gap between the eminentia mediana and the pars distalis, although its base on the gland has usually moved caudally and is found in the transitional zone between the cephalic (oral) and caudal (aboral) lobes in the adult. However, a few capillaries are present running from the brain wall to the aboral lobe or "segment postérieur" according to Grignon. They give rise to portal vessels not included in the porto-tuberal tract. They are described by Assenmacher and by Grignon and they are called the "veine porte postérieure" by the first-mentioned author. The posterior portal vessels and their forerunners are however in pronounced minority.

The conditions in the Sauropsida may be summarized as follows:

As shown by Wingstrand, the rostral part of the pars distalis, the cephalic lobe, is developed from the oral lobe of Rathke's pouch. The latter is recognized as that part of the pouch which is oral to the constriction of the lumen, from which the lateral lobes develop and to which the epithelial stalk adheres. In addition, the oral lobe is sometimes characterized by the development of a processus anterior. In the development of the vascular system another criterion of the oral lobe can be found: During the embryonic life it is more or less closely adhered to the part of the brain wall where the primary plexus will develop (the oral contact-zone), and it receives and takes up the capillaries which link the vascular plexus of the brain wall and of the adenohypophysial anlage. In other words, the forerunners of the portal vessels are destined to the oral lobe of Rathke's pouch.

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This contact between the hypophysial anlage and the brain is apparently attained mainly in two ways as far as can be concluded from available investigations. In *Lacertilia* and *Ophidia* and apparently also in *Chelonia* and *Rhynchocephalia* the original contact between the rostral wall of Rathke's pouch and the brain is locally retained by the development of the processus anterior of the oral lobe. In *Aves* and probably also in *Crocodilia* the original contact is lost, but it is reestablished when the cell buds of the rostral wall of the oral lobe grow on to the brain wall.

In this constantly occurring oral contact-zone the brain wall and the oral lobe are separated from each other by a thin layer of mesenchyme, the pial hypophysial mesenchyme of Wislocki. The linking capillaries traverse this layer.

In the *Mammalia,* Rathke's pouch is detached from the brain surface from the base of the pouch to the tip of the aboral region where the epithelium remains in contact with the brain wall of the saccus infundibuli in the aboral contact-zone (Fig. 27 D and E). The space between the pouch and the brain is filled with mesenchyme and during the development it is bordered laterally and orally by the growing lateral lobes. This type of early development of the adenohypophysial anlage is found in a comparatively great number of the investigated mammals, e.g. *Homo* (Atwell 1926), *Sus* (Salzer 1897, Haller und Mori 1925, Nelson 1933), *Bos* (House 1943), *Ovis* (Lubberhuizen 1931), some *Chiroptera* (Weber 1898), *Felis* (Brahms 1932), *Canis* (Kingsbury and Roemer 1940), *Mus, Sciurus, Tarsius, Macacus* (Woerdeman 1915), *Mus* (Kraushaar 1885), *Clethrionomys* (Enemar 1957), laboratory mouse (Kerr 1946), laboratory rat (Schwind 1928, Miraglia 1958), *Lepus* (Atwell 1918), *Dasypus* (Oldham 1941), some *Marsupialia* (Parker 1917). The attachment of the early anlage to the brain is pictured also by Gilbert (1935) concerning cat, calf, rat, pig, dog, and man. It is evident from the descriptions that the epithelial anlage of the hypophysis is not loosened from the brain wall to the same extent in all the said species. More or less temporary remaining attachments of median and oral portions of the pouch will be dealt with in the following section (p. 197).

In the present connection it is of great interest to note that the established mesenchyme-filled recess between the pouch and the adjacent part of the brain is rich in capillaries. The presence of the latter was noted by several authors studying the development of the mammalian hypophysis (Salzer 1898, Atwell 1918, Schwind 1928, Kingsbury and Roemer 1940, Kerr 1946), and the bundle of capillaries in question was named the pial-hypophysial plexus by Wislocki (1937) in his study on the development of the hypophysial vascular system in *Homo*. He showed that the pial hypophysial plexus embedded in the pial hypophysial mesenchyme played an important rôle in the rise of the sinus-system of the pars distalis, and his statements in this respect were confirmed by Glydon (1957) and by Enemar (1957) who used embryos from different rodents. The pial hypo-

physial plexus of Wislocki is the above mentioned linking capillaries between the prospective primary and secondary plexuses. The rise of the mesenchyme-filled recess and its boundaries was described in great detail by ATWELL (1918) in *Lepus* and (1926) in *Homo,* and therefore GLYDON (1957) calls the cavity in question Atwell's recess. This name will be adopted in the present investigation.

As pointed out in the preceding pages (p. 190) a conspicuous recess, the recessus transversus, is developed in the early hypophysial anlage also in several reptiles, above all in *Lacertilia* and *Ophidia.* However, this recess does not contain capillaries traversing from the plexus on the brain wall to the vascular system of the adenohypophysial anlage, according to the results obtained from injected embryos of the three reptiles investigated. This difference between the recessus transversus and Atwell's recess as regards their content of blood vessels suggested that they were not homologous structures. Therefore, the recess in the reptilian embryos was given a different name, recessus transversus (transverse recess), in the descriptions.

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The morphological difference between Atwell's recess and the recessus transversus is understood when the development of Rathke's pouch in *Mammalia* on one hand and in *Reptilia* and *Aves* on the other is compared according to WOERDEMAN (1915) and WINGSTRAND (1951). These authors showed that the rostro-oral part of Rathke's pouch, viz. the oral lobe of Wingstrand, does not develop into glandular tissue in any considerable degree in the *Mammalia,* but this is the case in the *Reptilia* and *Aves.* When the new characteristic of the oral lobe mentioned on p. 194 is considered, namely its relation to the brain wall in the oral contact-zone, it is of great interest to note that in the *Mammalia* the detached oral part of the pouch does not grow to reestablish the contact with the prospective eminentia mediana. The cleft between the brain and the pouch widens and forms Atwell's recess, it contains the pial hypophysial mesenchyme and the linking capillaries run free in the said mesenchyme for a comparatively long distance (Fig. 27 D, E). In the *Reptilia* and *Aves* the space corresponding to the Atwell's recess is occupied by the rostral portion of the oral lobe (the anterior process) leaving only very narrow space between the brain and the lobe. Consequently, the pial hypophysial mesenchyme is only a thin layer and the linking capillaries run free in the mesenchyme for a comparatively short distance (Fig. 27 A, B, C).

The difference between *Sauropsida* and *Mammalia* as regards the relation of the rostro-oral wall of Rathke's pouch to the anlage of the primary plexus and to the linking capillaries, and the difference between the said groups as regards the length and appearance of the linking capillaries is thus sufficiently explained and understood when a reduction of the oral lobe in the *Mammalia* is accepted. In this way the study of the anlage of the hypophysial portal system strongly supports the conclu-

sions drawn by Woerdeman and Wingstrand as to the homologies of the amniotic pituitary.

Atwell's recess consequently is a characteristic structure of the mammalian hypophysial anlage only. It is possible that the recess is of double origin, and that its aboral part is derived from a recessus transversus of the mammalian ancestor. It is however not certain that a conspicuous transverse recess ever existed, and it does not seem very probable that it should have been preserved in the mammals of to-day as a structure which could be identified. If so the transverse recess would be found as an unvascularized aboral part of Atwell's recess. It should however be borne in mind that a discussion of homologies based on the distribution of the capillaries in a mesenchyme-filled recess has its obvious limitations. The problem of the true nature of the aboral part of Atwell's recess however does not influence the principal conclusions in the comparative discussion of the pituitary homologies above.

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B. On epithelial rudiments in Atwell's recess.

The almost complete absence of a cephalic lobe in the hypophysis of *Mammalia* is a considerable difference from the structure of the hypophysis in the *Reptilia* and *Aves* when the following two features in the developing hypophysis are remembered. At first, the oral lobe is that part of the adenohypophysial anlage which receives the capillaries from the prospective primary plexus, and, secondly, the said linking vessels between the brain and the adenohypophysis are considered to be of the highest importance for the hypothalamic regulation of the activity of the pars distalis. Therefore, that part of Rathke's pouch in the *Mammalia* which receives the linking capillaries from the adjacent brain wall should be studied. The region in question is the caudal wall of Atwell's recess. Here a pronounced proliferation of the epithelium occurs, and the growing cell cords include among themselves the pial hypophysial capillaries which are transformed into the sinus-system of a large part of the developing pars distalis, as was first described by Wislocki (1937).

However, Atwell's recess is not always an undivided cavity. Very often a more or less conspicuous median ridge or process from the anterior wall of Rathke's pouch divides Atwell's recess into two cavities. Thus also the pial hypophysial plexus of capillaries, the linking capillaries, is divided into two bundles. This organization of the capillaries is found in *Homo* according to Wislocki's description and also in *Clethrionomys* (Enemar 1957). Atwell (1928) dealing with the development of the hypophysis in *Homo* describes two mesenchyme-filled recesses, named the "fossae". These structures are identical with the right and left halves of Atwell's recess, separated by a rostral process of Rathke's pouch containing a cavity which Atwell calls the anterior chamber.

The development of such an anterior process is easily studied in the bank-vole (*Clethrionomys glareolus*) and a brief description of the development of the

hypophysis in this species is given in an earlier paper (ENEMAR 1957). The rostral wall of Rathke's pouch in the early stages of the development is detached from the brain wall by the invasion of mesenchyme from each side of the pouch. This loosening from the brain does not continue to the midline. Therefore, a median part of the epithelium remains attached to the brain from the top of the pouch, the aboral contact-zone, down to the base of the pouch. This aboral-orally extended zone develops into a median ridge or evagination ("medialer Kamm" of Woerdeman, "crête médiane" of Weber) which is distinct from the lateral parts of the pouch. A transverse section through the pouch thus gives a T-shaped figure, where the rostral evagination is represented by the base of the T. A similar formation of an evagination attached to the brain occurs in *Felis domestica* (BRAHMS 1932, TILNEY 1914), in *Chiroptera* (WEBER 1898) and probably also in *Ovis aries* (LUBBERHUIZEN 1931). Median and rostral ridges of the anterior wall of Rathke's pouch facing Atwell's recess are found also in other mammalian species although they are not as distinct as in the species just mentioned, and although their development is not penetrated in detail. The following species seem to belong to this category: *Sus* (HALLER and MORI 1925, SALZER 1898), *Sciurus* and *Tarsius* (WOERDEMAN 1915), and *Homo* (ATWELL 1926). However, such rostral evaginations seem to be completely absent or very indistinct in other species, e.g. *Lepus* (ATWELL 1918), *Mus musculus* (KERR 1946), *Mus decumanus* (WOERDEMAN 1915, SCHWIND 1928), and *Apodemus flavicollis* and *Sorex araneus* (author's collection).

The development of the rostral evagination has been followed in detail in *Clethrionomys* (Fig. 27 D and Fig. 28 A—D). In the earliest stages it seems to be a rostro-oral prolongation of the epithelium in the aboral contact-zone, i.e. the anlage of the pars intermedia. As in the latter the epithelial arrangement of the cells is retained in the rostral evagination. It is surrounded laterally and orally by the pial hypophysial mesenchyme with the linking capillaries. In later stages the rostral evagination is separated from the epithelial layer of the intermedia anlage by a transverse furrow (Fig. 28 C). During the development of this structure the rostral evagination is completely surrounded by mesenchyme, but its rostral tip is still in contact with the brain wall. This contact is located to the region where the prospective primary plexus is to be found, and the linking capillaries run along the rostral evagination in the Atwell's recess on to the proliferating epithelium of the pouch. The cells of the evagination show a strict epithelial arrangement, but the lumen no longer communicates with the lumen of Rathke's pouch. In the following stages (Fig. 28 D) the rostral evagination loses its contact with the brain wall, but it can still be found in later stages as a rudimentary epithelial cyst among the proliferating cell cords in Atwell's recess. At last it seems to dissappear completely.

It can be added to the descriptions above that in the embryo of *Lemmus lemmus*, 12 mm in length (CR), (author's coll.), a similar rostral evagination as

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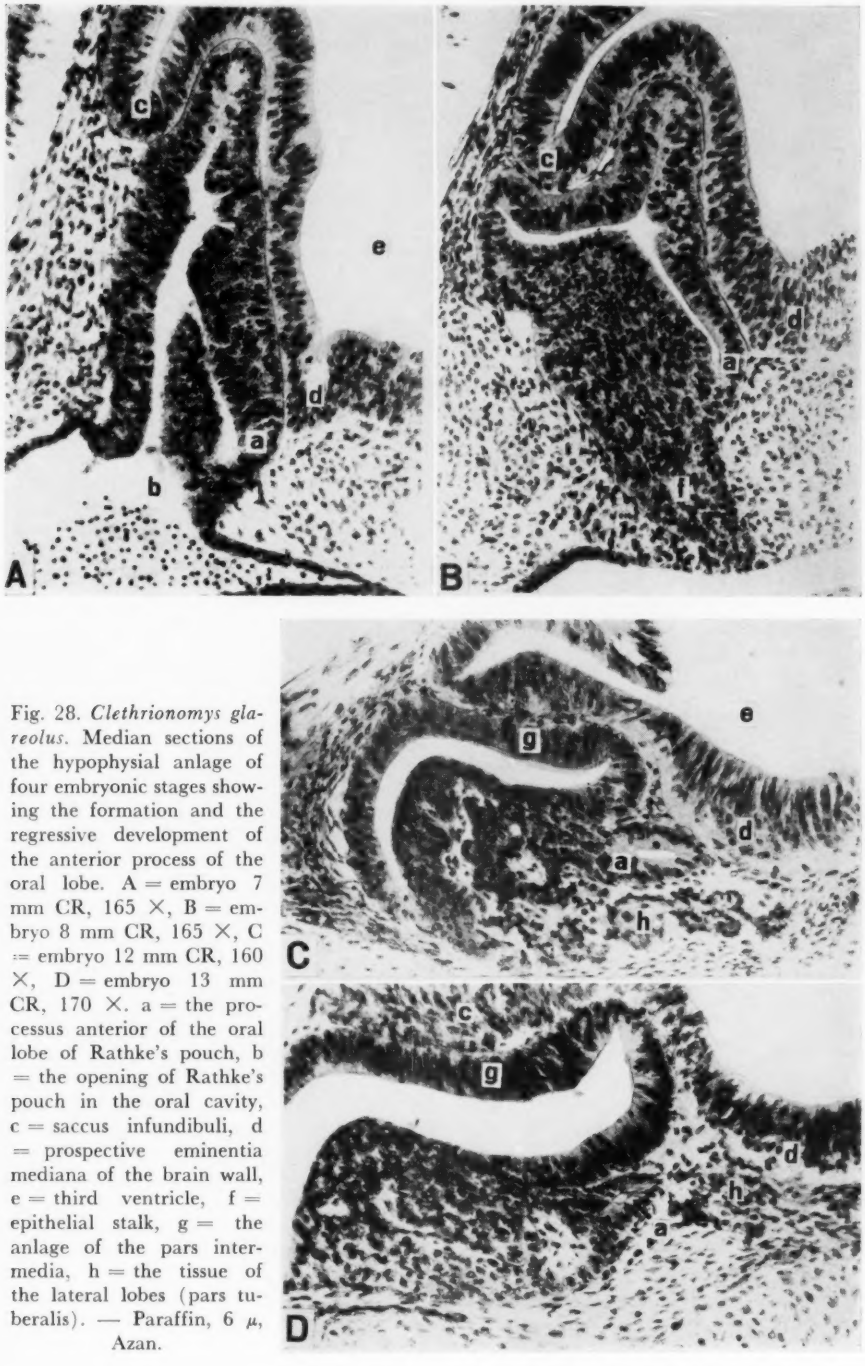

Fig. 28. *Clethrionomys glareolus*. Median sections of the hypophysial anlage of four embryonic stages showing the formation and the regressive development of the anterior process of the oral lobe. A = embryo 7 mm CR, 165 ×, B = embryo 8 mm CR, 165 ×, C = embryo 12 mm CR, 160 ×, D = embryo 13 mm CR, 170 ×. a = the processus anterior of the oral lobe of Rathke's pouch, b = the opening of Rathke's pouch in the oral cavity, c = saccus infundibuli, d = prospective eminentia mediana of the brain wall, e = third ventricle, f = epithelial stalk, g = the anlage of the pars intermedia, h = the tissue of the lateral lobes (pars tuberalis). — Paraffin, 6 μ, Azan.

in the 12 mm embryo of *Clethrionomys* is found. Since no embryos of other stages were available the rise and further development of the observed process could not be followed, but it is very probable that the structure in question is of the same nature as in *Clethrionomys*.

It is of the greatest interest to read WEBER's (1898) description of the development of his "crête médiane" in the anlage of the adenohypophysis in *Chiroptera*. He declares that this structure is reduced in later stages. Perhaps also some structures in the illustrations of the pituitary development in *Felis* (BRAHMS 1932), e.g. plate 2:9, might be interpreted as rudiments of the rostral evagination undoubtly present in earlier stages in this species, but this conclusion remains uncertain.

When trying to explain the morphological significance of the rostral and median evaginations described above the first possibility suggesting itself is to homologize it with the processus anterior of the oral lobe in the general scheme of the amniotic adenohypophysial anlage. If this interpretation is right then the described development of the rostral evagination in the mammalian hypophysial anlage constitutes another support to Woerdeman's and Wingstrand's view that the oral lobe remains rudimentary in the mammalian hypophysis.

The developing mammalian hypophysis shows many variations between the species as to the general shape of the anlage, its evaginations and mode of proliferation. Therefore, comparisons should be made with great care. The goal of a comparison in the present connection is to trace, if possible, the boundaries of the oral lobe of Rathke's pouch. In comparison with the adenohypophysial anlage in the *Sauropsida* considerable difficulties arise as to the mammalian anlage due to the fact that the attributed characteristic of the oral lobe listed on p. 146 can not always be identified. The lumen of Rathke's pouch does not always show a clear oral dilatation separated by a constriction from the dilated lumen of the aboral lobe. The point of origin of the lateral lobes cannot be exactly determined as the lobes are budded off as solid epithelial masses from a very broad zone near the base of the pouch. Therefore, it is not easy to decide whether the rostral evagination belongs to the oral or to the aboral lobe.

The fact that the rostral evagination in the described rodents shows a close relation to the intermedia anlage during a considerable time of the embryonic life and that the histology of the two structures is similar supports the view that the rostral evagination should be referred to the aboral lobe. However, these similarities between the two structures might be a consequense of their common contact with the brain wall only. In any case, similarities in the histology cannot always be used as criteria of decisive importance when studying the homologies in the pituitary.

Instead, the following features show that the rostral evagination must be an anterior process of the oral lobe (see Fig. 27 A—D):

1. The epithelium very near the base of the early Rathke's pouch is the main

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contributor to the formation of the process, and this mode of development is exactly similar to that which in *Gallus* results in the anlage of the rostral part of the oral lobe, according to WINGSTRAND's interpretations (1951, Fig. 56).

2. The rostral tip of the process remains more or less closely attached to the area of the brain wall where the prospective primary plexus is located.

3. The process is always situated in Atwell's recess and is thus surrounded by the pial hypophysial mesenchyme and the linking capillaries.

The significance of the last two points as attributes to the oral lobe of the adenohypophysial anlage was analysed above (p. 194). In the author's opinion the three points listed above are sufficient to settle the rostral evagination in the developing hypophysis of *Clethrionomys* as being homologous to the processus anterior of the oral lobe in the *Sauropsida*.

The ontogenetic observations in the present chapter indicate that the adenohypophysial anlage in *Reptilia* and *Mammalia* is differentiated according to a common pattern, also with regard to the anterior process, although the latter is insignificant in *Mammalia*. When an anterior process is clearly laid down in the mammalian embryo it shows a regressive development and it does not contribute to the formation of the pars distalis to any considerable extent. These facts agree with the statements of Woerdeman and Wingstrand that the oral lobe is vestigial in *Mammalia*.

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The development of the hypophysial portal vessels.

The portal vessels are identified as a bundle of relatively long and unbranched vessels of larger calibre linking the primary and secondary plexuses. The development of the portal vessels is often said to be combined with dislocations of different parts of the hypophysial anlage all of which have in common that the primary and secondary plexuses are separated from each other. These processes will be discussed below.

In the last few years other types of portal vessels have been described in some mammals running from plexuses on the aboral surface on the infundibular stem or from the vascular system of the neural lobe (XUEREB, PRICHARD and DANIEL 1954; PRICHARD and DANIEL 1956, 1957; MIRAGLIA 1958). These "short" portal vessels are not included in the discussion below.

The formation of the portal vessels does not mean that the vascular contact between the eminentia mediana and the pars distalis is established for the first time. This contact exists already from the first appearance of vessels in the pituitary anlage, when the adenohypophysis is represented by the open pouch of Rathke. The direction of the blood stream is also from the beginning towards the hypophysial anlage in the linking vessels, as stated for the birds and mammals investigated, and this is also true for the reptiles in the present investigation. The linking capillaries are designated as small veins (veinule pie-mérienne) by

ASSENMACHER (1952) and GRIGNON (1956) in birds. In all cases the estimation of the direction of the blood flow is concluded from the arrangement of arteries and veins in the pituitary region. GLYDON (1957) could not say anything definite about the direction of the blood flow in and around the developing pituitary until at a very advanced stage in the embryo of the laboratory rat (seventeen days after insemination). This is explained by the fact that he misinterpreted the oral drainage along the epithelial stalk as being a supply to the pituitary anlage.

As regards the reptiles the appearance of the portal vessels can be briefly recapitulated from the descriptions as follows. In *Lacerta* the anlage of the adenohypohpysis is dislocated caudally in the 55 mm embryo (p. 153). This means about a week before hatching under the conditions described above (p. 144). By this dislocation the rostral end of the oral lobe is of course drawn away from the oral contact-zone and thereby the linking vessels are stretched out and appear as portal vessels between the primary and secondary plexuses (Fig. 27 A). In *Anguis* such an obvious moving of the anlage of the adenohypophysis never occurs. In the 60 mm embryo (p. 167) it is possible to identify portal vessels. A considerable amount of mesenchyme has invaded the oral contact-zone, and the rostral end of the oral lobe is also somewhat excavated. The linking capillaries now traverse the thickened mesenchymal layer and being of relatively large calibre they are designated as portal vessels. In addition, in the following stages, embryo 70 mm (TL), the oral lobe is slightly removed from the eminentia in caudal direction and the portal vessels of course become more prominent. In *Natrix* the formation of typical portal vessels seems to be restricted to the last week before hatching. It is obvious that the long portal vessels appear when the cephalic lobe of the pars distalis is dislocated laterally. Consequently, the portal vessels are drawn to one side, either to the right or to the left, and thus they contribute to the asymmetric organization of the pituitary in the snake (Fig. 27 B).

According to the detailed descriptions by WINGSTRAND (1951), ASSENMACHER (1952), and GRIGNON (1956) the rise of the portal vessels is mainly the same in birds. The adenohypophysis is dislocated from the eminentia in oral or oral-caudal direction (Fig. 27 C). This process is very conspicuous in birds and the resulting space between the eminentia and the pars distalis is comparatively wide. The portal zone (Wingstrand) of the pars tuberalis adheres to the outstretched vessels which bridge the said space, and the vessels, together with the tissue of the pars tuberalis, constitute the porto-tuberal tract (tractus porto-tubéral, Assenmacher). This tract is completely surrounded by the subarachnoid space in most species, with the exception of the bird *Elaenia albiceps,* in which the pars tuberalis and the portal vessels seem to have a mammal-like position according to Wingstrand.

In mammals the conditions for the formation of the portal vessels are not the same, since there is no oral lobe covering the primary plexus as in reptiles and birds (Fig. 27 D and E). Thus there is no extended area of contact between the

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primary and secondary plexuses in mammals like that found in the oral contact-zone of reptiles and birds. In *Clethrionomys* and *Apodemus* (ENEMAR 1957) the portal vessels appear more gradually between the plexuses. They are formed from linking capillaries in a very narrow zone on the caudal border of the primary plexus. GLYDON (1957) considers that in the rat the development of the infundibular stem means a distension of the distance between the primary and secondary plexuses with the appearance of the portal vessels as a consequence. Such a process could not be stated in *Clethrionomys* and *Apodemus*. In the investigated mammals the portal vessels do not form a separated tract surrounded by the subarachnoid space, but they lie together with the tissue of the pars tuberalis close to the oral surface of the infundibular stem. There are exceptions from this rule. A bird-like porto-tuberal tract is developed in *Cetacea* (HARRIS 1947, 1950), and *Monotremata* (HANSTRÖM and WINGSTRAND 1951), whereas in some mammals there is a relatively close contact between the primary and secondary plexuses without a conspicuous bundle of long an unbranched portal vessels (DE GROOT 1952).

The development of the primary plexus.

A detailed investigation on the development of the primary plexus of the hypophysial portal system has not yet been carried out. Some information has however accumulated during the study on other parts of the hypophysial vascular system, especially the portal vessels.

In some investigations (e.g. HARRIS 1955, GLYDON 1957) the name primary plexus is used for the capillary loops only. The latter are curved vessels penetrating the tissue of the eminentia mediana, and they are usually developed from the plexus of capillaries on the surface of the eminentia. These loops are a specialization found in mammals, but the functional properties ascribed to these loops of course are valid also for the superficial plexus of capillaries as pointed out by several authors. As is clear from the descriptions the name primary plexus is applied to the total vascular plexus of the eminentia mediana in the present investigation.

The primary plexus is evidently differentiated comparatively early during the embryonic life in the investigated reptiles. In *Lacerta,* in which the arrangement of the primary plexus can easily be studied, the capillary plexus on the prospective eminentia has become dense already at the 33 mm stage and has attained the main organization of the primary plexus of the adult, i.e. after 12 days of development under laboratory conditions and 11 days before hatching. Also in *Anguis* and *Natrix* the primary plexus has become a dense network of capillaries relatively early, but it was not possible to state its formation with the same accuracy as in *Lacerta.* It was however characteristic also of these two species that the primary plexus was formed long before the appearance of the typical portal vessels.

In the early embryo the oral part of the diencephalon has a rich capillary plexus on its surface in all three species, and this plexus is supplied by the infundibular arteries. The arrangement of the capillaries shows a characteristic pattern since a number of the vessels converge towards the oral contact-zone and meet there, and here the congregation of capillaries constitutes the anlage of the primary plexus. In later stages the superficial capillaries on the hypothalamus are partly reduced, and the few remaining converging capillaries give rise to the main supplying vessels to the primary plexus. In the area of the oral contact-zone no reduction of vessels occur, but the accumulation of capillaries here is transformed into the primary plexus. This process is valid for *Lacerta* and *Anguis*, and also for *Natrix* though some modifications are found in this species. The arrangement of converging capillaries seems to appear later in this species and the said reduction of them also occurs later compared with the corresponding stages of the development of the hypophysis in *Lacerta* and *Anguis*. Judging from the morphology of the vascular plexus on the wall of the tuber cinereum the primary plexus is functional long before the rise of typical portal vessels, i.e. already during the period when ordinary capillaries link the primary and secondary plexuses in the oral contact-zone.

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In birds the capillary plexus of the oral contact-zone becomes dense at a very early stage of the hypophysial development. Assenmacher (1952) observed a very dense capillary network in *Anas* already in the stage at which the formation of the secondary plexus started, i.e. long before the rise of the typical portal vessels. Grignon (1956) states that the primary plexus is developed in the days after the fifth day of incubation in *Gallus*. This is in line with Assenmacher's observations.

Also in the rodents (Enemar 1957, Glydon 1957) the primary plexus is distinct before the development of the portal vessels. However, at this stage the primary plexus is represented by a dense superficial network of capillaries on the eminentia. The capillary loops are not formed until after birth in the investigated rodents. These loops of course make the primary plexus more efficient, but their formation does not coincide with the onset of the function of the primary plexus. The dense capillary network formed several days before birth may also function. These problems will be discussed in another paper.

When studying the differentiation of the primary plexus in mammals one would expect the rise of the said plexus to vary considerably in time when compared with the time of birth, due to the fact that the gestation periods are not biologically the same from one species to another. Niemineva (1954 a, b) gives some information of the development of the primary plexus and portal vessels in the human embryo. Here the capillary loops are formed during the latter half of the embryonic life. In the investigated rodents referred to above the young are born "early", hairless and blind. Another timecourse in relation to the gestation period will probably be found for the development of the hypophysial vascular system for rodents whose young are born at a more advanced stage.

The development of the hypophysial vascular system

In *Lacerta* and *Anguis* the primary plexus undergoes only few changes after its formation (Fig. 6). It remains as a simple and flat network of capillaries on the surface of the eminentia. In *Natrix* the contact surface between the primary plexus and the tissue of the eminentia is more intimate as the capillaries are sunk into the deep furrows which are formed gradually in the period after hatching. Though these capillaries give rise to a complicated pattern of vessels in the eminentia they do not penetrate the eminential tissue in *Natrix* as do the capillary loops in mammals (compare in this respect DIEPEN 1955). These capillary loops are probably a secondarily attained specialization in the higher tetrapods. This view is in accordance with the statements made by HANSTRÖM (1953) on the basis of comparative studies on the mammalian primary plexus and eminentia mediana.

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The relation of the lateral lobes to the development of the hypophysial portal vessels and the primary plexus.

Though the pars tuberalis in the adult shows histological signs of a secretory activity (ROMEIS 1940, WINGSTRAND 1951) no endocrine function has been demonstrated (HARRIS 1955). Its rôle in the pituitary activity seems to be of negligible importance. Therefore, it has been attempted to find some function of the tuberal lobe or its anlage, the lateral lobes, during the embryonic life.

HARRIS (1947) assumes that the purpose of the tuberal lobes is to establish the portal vessels. WINGSTRAND (1951) found no support for this view in the development of the vascular system of the chick. In birds the portal vessels appear independently of the lateral lobes which secondarily adhere to the portal vessels already formed. HARRIS (1955) returned to the problem and although he adopts Wingstrand's statements he concludes that "it is clear that there is some close relationship between the pars and zona tuberalis and the portal system". GRIGNON (1956) also discussed the possible rôle of the lateral lobes on the basis of his studies on the development in the chick. He observed that the developing portal vessels in the oral contact-zone were found mainly in the lateral parts of the zone, i.e. in the immediate neighbourhood of the lateral lobes. Therefore, he thinks that it is possible at least to ascribe to the lateral lobes "un rôle de vecteur dans la développement des vaisseaux portes".

It is apparent that the choice of the species for the embryological study greatly influences the investigator's opinion on the rôle of the lateral lobes. Harris studied the mammal *Lepus,* and it is true that in the *Mammalia* there is a close relationship between the developing portal vessels and the lateral lobes. It is shown for *Clethrionomys* and *Apodemus* (ENEMAR 1957) that the linking capillaries are located in a furrow formed by the lateral lobes and covered by the infundibular stem in the developmental stage before they are transformed into portal vessels.

In birds this contact is not so clear although Grignon found some relation between the lateral lobes and the developing portal vessels.

The study of the development in reptiles gives clear information in this respect. As described above the lateral lobes are never developed in *Natrix*, and consequently no tuberal lobes are formed. In spite of this a bundle of typical portal vessels is formed. In *Lacerta* and *Anguis* conspicuous lateral lobes grow out from the hypophysial anlage and also reach the surface of the tuber cinereum. They however never show any clear relation to the portal vessels. In the oral contact-zone the linking capillaries are found especially in the center. A concentration of the vessels to the neighbourhood of the lateral lobes could not be detected. Therefore, Grignon's supposition can not be applied to *Lacerta* and *Anguis*.

If we wish to study the possible rôle of the lateral lobes in the development of the portal vessels as many species as possible should be examined. If we only consider the three amniotic classes the experience from these three investigated reptiles shows the complete absence of a relationship between the said structures. Only a doubtful relation is found in one investigated bird. As regards the mammals the clear relationship between the structures may be secondarily attained. We should remember that the structure of the region where the portal vessels are formed in *Mammalia* is not strictly homologous with the corresponding space in *Aves* and *Reptilia*. The processus anterior of the oral lobe is reduced, and the consequences as to the structure and course of the linking vessels preceding the portal vessels were discussed above (p. 196). It is also characteristic of the development of the mammalian pituitary that the lateral lobes are relatively large structures when compared with those of birds and reptiles.

Summing up, it is difficult to find a good support for the supposed action of the lateral lobes in the development of the portal vessels when the embryology of members of the three amniotic classes is studied and compared. Therefore, it seems to be most reasonable to abandon the formation of the portal vessels when searching for the significance of the pars tuberalis or its anlage.

The pars tuberalis when present in the adult usually covers the eminentia mediana with its primary plexus of the hypophysial portal system, sometimes as a collar also surrounding the infundibular stem. Exceptions are the lizards *Lacerta* and *Anguis* where the tuberal lobes are included in the brain wall aborally to the eminentia mediana in the sulcus infundibularis. The effect of the lateral lobes on the development of the primary plexus has therefore been discussed by some French scientists. Assenmacher (1952) and Benoit and Assenmacher (1955) state that the primary plexus in *Anas* is formed already before the lateral lobes reach the eminentia mediana. Grignon (1956) agrees as to *Gallus* and concludes that all influences of the lateral lobes on the development of the primary plexus should be denied. The observations obtained from the reptilian material in the present investigation support this view. Turning to the mammals the same condition as to the relation of the lateral lobes to the developing primary plexus is

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not found. As shown for the embryo of the bank-vole (ENEMAR 1957) the lateral lobes reach the prospective eminentia mediana in the 10—12 mm (CR) embryo. From about the same stage the capillary plexus on the eminentia begins to become denser, but it is not very dense until in the 16 mm embryo. The same is true also for the laboratory mouse in which the development of the primary plexus has been more closely studied (ENEMAR in preparation). Thus there is a marked difference between the reptiles and birds on one hand and the mammals on the other.

Also in this connection the homologies of the amniotic pituitary should be applied. In reptiles and birds the primary plexus develops between the prospective eminentia mediana and the oral lobe, i.e. in the oral contact-zone. The primary plexus is formed long before the oral lobe withdraws from the eminentia as is clear from the descriptions of the three reptiles in the present investigation. Also in birds the primary plexus becomes dense very early. In other words, it is not possible for the lateral lobes to make contact with the eminentia until the oral lobe has moved away, and on this occasion the lateral lobes have to adhere to an already differentiated primary plexus. In mammals, however, no oral contact-zone is established after the epithelium of Rathke's pouch has loosened from the prospective eminentia since the oral lobe remains vestigial as described above and thus it is possible for the lateral lobes to grow on to the surface of the latter. And so they do, and the primary plexus is then differentiated between the prospective eminentia and the tuberal lobe, also in this case in a contact-zone, "die proximale Adeno-neurohypophysäre Kontaktfläche" of SPATZ (1955).

The question is whether the presence of adenohypophysial epithelium on the prospective eminentia is necessary for the development of the dense primary plexus. If so, the development of the comparatively large lateral lobes and their early adherence to the prospective eminentia has a developmental significance in *Mammalia*. In other words, the lateral lobes act as a substitute for the oral lobe in the establishment of the contact between the adenohypophysial anlage and the prospective eminentia. In this connection the behaviour of the lateral lobes in *Lacerta* and *Anguis* should be remembered. Here the lateral lobes are attached to the lateral surface of the tuber cinereum (p. 152, Fig. 4 and 10) but without the development of a vascular plexus in between. Instead, it was pointed out that the contact-zone remained completely unvascularized. Maybe, the answer is that the development of the primary plexus is a result of an interaction between the lateral lobes and the prospective eminentia. This question can be solved only by means of experiments. The significance of the contact between different parts of the hypophysial anlage for the differentiation of special structures should not be overestimated. Such relationships have been studied by means of experiments especially in amphibians. It was found and accepted for a long time that the presence of the epithelium of Rathke's pouch was necessary for the development of the saccus infundibuli (see BLOUNT 1945 and others). According

to EAKIN and BUSH (1957) a neural lobe with normal histology developed in two frog species in spite of the complete absence of the epithelial anlage of the hypophysis. As to the eminentia mediana the results varied greatly. In some specimens the eminentia appeared to be normal, in others it was abnormal and showed a membranous character. The vascular system was not discussed.

The development of the vascular system of the pars distalis and the neural lobe.

The vascular system of the pars distalis and the neural lobe in the three reptiles in the present investigation is formed in the same way as in birds and mammals. When the anlage of the pars distalis proliferates it includes the surrounding mesenchyme with its content of capillaries, and the latter are transformed into the sinusoids of the secondary plexus. As stated for mammals (WISLOCKI 1937, GLYDON 1957, ENEMAR 1957) the anterior wall of Rathke's pouch (the caudal wall of the Atwell's recess) shows a very intensive proliferative activity in the formation of the pars distalis. This is of course not the case in reptiles and birds in which the oral lobe of the anlage is retained and develops to the cephalic lobe of the pars distalis. In the last-mentioned two classes the proliferative activity seems to be more equally distributed over the epithelial anlage, of course with the exception of the anlage of the pars intermedia. It was however observed that in *Lacerta* and *Anguis* the aboral lobe formed the main portion of the pars distalis, whereas in *Natrix* the oral and aboral lobes gave rise to lobes of about equal size. The proportions can not be exactly stated as it is difficult to hold the derivatives of the two lobes apart during the development.

The rise of the vascular system of the neural lobe is a very simple process in the three reptiles studied. The original capillary plexus on the saccus infundibuli is retained and enlarged during the development, but it always remains superficial. In *Natrix* the vessels are included in deep furrows which are formed when the saccus proliferates and establishes the neural lobe.

It seems to be a common observation that the circulation of the neural lobe is separated from the hypophysial portal circulation in the adult amniote. These statements are reviewed by DE GROOT (1952) and BENOIT and ASSENMACHER (1955). This separation manifests itself in the establishment of a separate arterial supply to the neural lobe and in the lack or scarcity of vessels linking the secondary plexus with the vascular system of the neural lobe. In the early anlage of the vascular system of the hypophysis the capillaries of the anlage constitute an anastomosing unit. In the investigated reptiles the recessus transversus seems to be the structure, which partly separates the said circulations from each other. In the recess a connective tissue sheath is developed, and as mentioned in the descriptions, this sheath is not traversed by any capillaries. The original communications however persist in two regions. Vessels always connect the vascular system of the neural lobe with the superficial vessels on the thin pars intermedia, and capillaries

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running on the oral surface of the infundibular stem connect the primary plexus with the neural lobe. The direction of the blood flow in the first mentioned vessels could not be estimated with certainty. Since the vena retrohypophysea is located aborally to the neural lobe and the pars intermedia, the linking vessels may be used as a draining path for the blood from the pars distalis beside the direct communications with the vein. In the capillaries on the infundibular stem the blood flows to the neural lobe judging from the arrangement of the arterial supply to the primary plexus and the neural lobe during the development. This is true at least for *Lacerta* and *Anguis* whereas it has not been possible to clear up these conditions with certainty in *Natrix*.

In mammals the thick pars intermedia is said to establish this separation, and if the pars intermedia is not developed it is replaced by a lamina of connective tissue. However, detailed investigations on the vascular system of the hypophysis in some mammals have unveiled "overflows" and "short portal vessels" which link the two circulatory systems in question, and it must be admitted, that we do not yet know very much about the occurrence and significance of these vascular communications. At least it is not yet possible to give a general characterization of this structure in the *Amniota*.

The Drainage

The main venous outlets from the hypophysis are laid down very early during the development, and they show only insignificant changes in the development up to the adult stage. The main venous system of the developing head, however, undergoes great changes in the *Amniota,* and in the present investigation the possible pathways of the hypophysial blood from the head has been studied during the embryonic life and in the adult.

There are only few investigations on the venous outlets from the hypophysis outside the sella turcica. In order to make comparisons as to the hypophysial venous system in the *Amniota,* it is necessary to study the structure of the venous pattern also relatively far from the pituitary. It appears however that as a rule information is scarce in the literature on the development of the venous system in the basal parts of the head, with the exception of a few species, as e.g. *Natrix*. A comprehensive and comparative work as that by VAN GELDEREN (1924, 1925) completely left out of consideration the veins in the cranial base, as for instance the large anastomoses there. This circumstance may cause some trouble as to the nomenclature of the observed veins in the present investigation. The author has however avoided as far as possible to introduce new names since this is beyond the scope of this work. A revision in this respect must be based on a broad comparative study on the venous pattern of the cranial base and such an investigation has not yet been carried out.

DESCRIPTIONS

Lacerta agilis

The development of the main lateral veins in the head was described already by GROSSER and BREZINA (1895) in this species and SHINDO (1915) contributes to the knowledge of the developing veins also in the pituitary region. Also VAN GELDEREN (1924) gives som information about the development of the veins draining the brain. BRUNER (1907) published a very detailed description of the venous system in the head of the adult. His statements are fully confirmed by the present investigation and has greatly facilitated the analysis of the developing venous system together with the main results of Grosser and Brezina and of Shindo.

E m b r y o 4 m m C R. The basic pattern of the venous system of the head is already established at this stage. Venous channels from the forebrain and the draining vessels from the anlage of the sinus orbitalis run together and pass the hypophysial region as a single vena capitis medialis. This vein continues ventromedially to the ganglion Gasseri. Thereupon it curves laterally and passes the lateral wall of the anlage of the auditory organ and the remaining cranial nerve roots, and it is now named vena capitis lateralis. In the pituitary region the left and right vena capitis medialis anastomose by way of the vena retrohypophysea, described already by SHINDO (1915). After leaving the vena capitis medialis the vena retrohypophysea passes orally to the profundus ganglion and aborally to the carotis interna before reaching the hypophysial anlage. Here it is located immediately aborally to the tip of the aboral lobe, and it is attached to the brain wall, approximately to the area where the saccus infundibuli develops later on. The vena retrohypophysea is connected with the capillary system of the hypophysial anlage and the adjacent brain wall, and thus acts as the outlet of the blood of the pituitary region. No other draining pathways from this area were found at this early stage.

E m b r y o s 5,5 m m C R—28 m m T L. In the first stages of this period a new median vessel in the plica encephali ventralis appears. It runs orally from the aboral region of the plica and takes up vessels from the hypothalamic wall and finally empties into the vena retrohypophysea just above the tip of the hypophysial anlage (Fig. 29).

The venous pattern of the hypophysial region in relation to the main anatomical structures in the 28 mm embryo is shown in Fig. 30. The vena retrohypophysea collects all blood leaving the hypophysial anlage. The vein enters the pituitary region via the fenestra metoptica just in front of the pila antotica and passes close aborally to the hypophysial anlage. At this stage also another anastomosis is formed, namely between the left and the right sinus orbitalis. It is the vein which is named the vena anastomotica by SÄWE-SÖDERBERGH (1946) and it is located to the anterior margin of the fenestra metoptica below the subiculum

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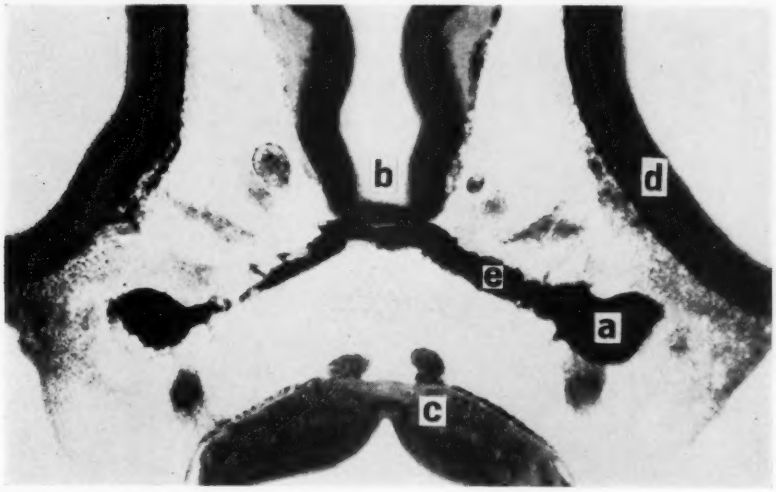

Fig. 29. *Lacerta agilis*. Embryo 5,5 mm CR. Horizontal section just aboral to the Rathke's pouch showing the vena retrohypophysea (e) at this early stage. — a = vena capitis medialis, b = the third ventricle of the diencephalon, c = myelencephalon, d = the anlage of the retina. — Incomplete ink injection, celloidin 60 μ, Mallory's phosphostungstic acid haematoxylin, blue filter, 75 ×.

infundibuli. The space in the fenestra between these two veins is filled up by the retractor bulbi group of eye muscles. They enter the pituitary region and are inserted on the trabeculae cranii laterally to the hypophysial anlage.

During this period a new communication is developed between that part of the vena cerebralis media which leaves the cranial cavity together with the nervus trigeminus, and the vena capitis medialis. It joins the latter near the vena retrohypophysea. This vein was described by Grosser and Brezina (1896) as vena cerebralis media secundaria. In later stages this vein and the vena retrohypophysea have a short common stem emptying in the vena capitis medialis, which is the permanent condition in the adult (cf. also Bruner 1907).

Embryo 40 mm TL. This stage is pictured in Fig. 31. A vein appears in the bottom of the sella, running between the ventro-caudal margin of the adenohypophysial anlage and the crista sellaris. It is connected with the vena retrohypophysea via two branches near the lateral flanks of the hypophysis. This new vein, in the present investigation called the vena intrasellaris transversus, leaves the sella together with the internal carotid through the carotid foramen. It then follows the carotid only for a short distance, and thereafter, curving laterally, joins the vena capitis medialis. A few thin branches can also be followed along the carotid on to the region of the facialis ganglion where they join the vena capitis lateralis. The vena intrasellaris transversus takes up one or two veins from the oral roof behind the sella. These veins enter the sella through the foramen

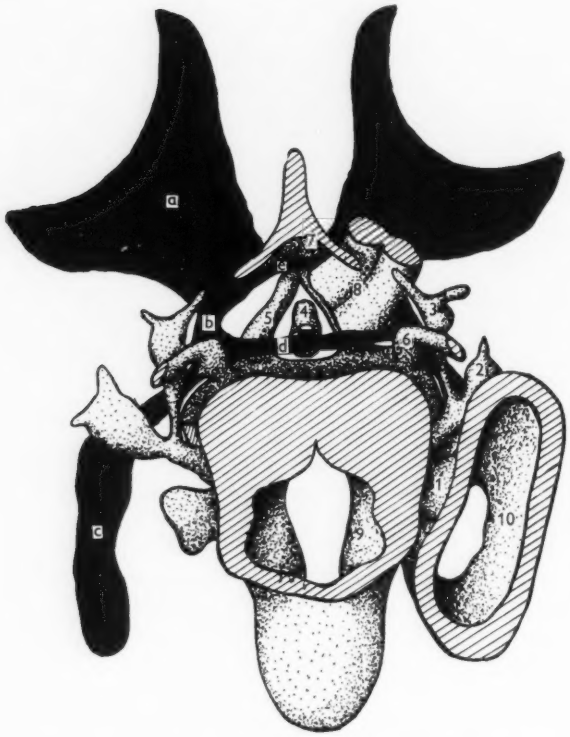

Fig. 30. *Lacerta agilis*. Embryo 28 mm TL. Drawing showing the relation of the veins to the main anatomical structures in the base of the head. Dorsal view. Cut surfaces are obliquely hatched. 1 = acustico-facialis ganglion, 2 = ganglion Gasseri, 3 = profundus ganglion, 4 = adenohypophysial anlage, 5 = trabecula cranii, 6 = pila antotica, 7 = subiculum infundibuli and pila metoptica, 8 = retractor bulbi muscles, 9 = rhombencephalon with the fourth ventricle, 10 = otic capsule. The veins are drawn in black. a = sinus orbitalis, b = vena capitis medialis, c = vena capitis lateralis, d = vena retrohypophysea, e = vena anastomotica. The drawing is based on a reconstruction made by projecting camera lucida drawings of a number of sections over each other. The embryo was ink-injected, embedded in celloidin, and cut 60 μ. Diencephalon is excluded from the reconstruction and also the otic capsule and the retractor bulbi muscles of the left side.

cranio-pharyngeus. No direct communications were found between the vascular system of the hypophysial anlage and the vena intrasellaris transversus.

Embryos 55 mm TL—65 mm TL (the hatching stage). There are only few changes in the venous system surrounding the hypophysis compared with the preceding stage. The hypophysis is now laterally and orally embraced by the retractor bulbi muscles. The crista sellaris is ossified, and its internal blood rooms are in communication with the vena intrasellaris transversus on several points (Fig. 7). The vena retrohypophysea is the main outlet for the blood from the hypophysis. It is still located aborally to the gland and covers the dorsal

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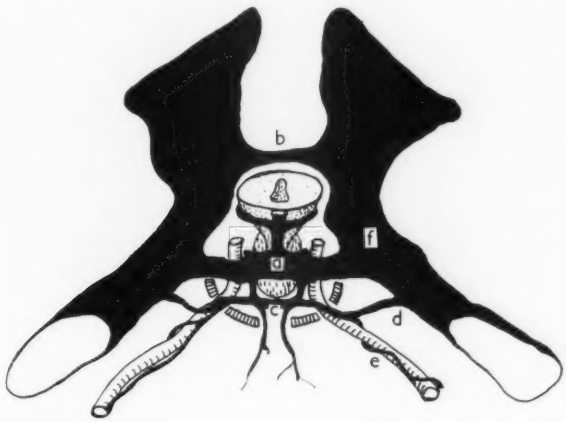

Fig. 31. *Lacerta agilis*. Embryo 40 mm TL. Semidiagrammic drawing showing the arrangement of veins in the pituitary region. Dorso-caudal view. The oral half of the hypothalamus (stippled) and the hypophysis (short vertical lines) are included in the drawing. The vena retrohypophysea (a) is seen to take up a single median vein draining the hypothalamus and the plica encephali ventralis. The two lateral communications between the vena retrohypophysea and the vena intrasellaris transversus (c) are shown. The latter vein communicates with the head vein (at d) via the carotid foramen and it also takes up a few venous twigs via the foramen cranio-pharyngeus (near c). Some small veins from the system of the vena intrasellaris follow the internal carotids (e). — b = vena anastomotica, f = vena capitis medialis. The limits of the sella are indicated by striated columns. The drawing is based on the same reconstruction technique as in Fig. 30.

aspect of the latter. It has several communications with the vascular plexus of the neural lobe. The sinus-system of the pars distalis empties mainly into the venous channels which connect the vena retrohypophysea with the vena intrasellaris transversus. The blood from the portal vessels flows through the sinus-system of the cephalic lobe to the sinus-system of the caudal lobe before reaching the venous outlets from the hypophysis.

The adult stage. In two ink-injected and celloidin-sectioned adult heads of the species the only existing venous outlet from the hypophysial vascular system that could be demonstrated was the vena retrohypophysea. It traverses the pituitary region dorsally to the gland and just in front of the anterior edge of the dorsum sellae. The vein is very wide, and it is therefore partly located in the sella between the dorsum and the gland. The hypophysial blood enters the vena retrohypophysea from the vascular plexus of the neural lobe and from the sinusoids of the caudal lobe and the pars intermedia of the adenohypophysis. The presence of venous outlets from the sella via the foramen caroticum from the vena intrasellaris could not be evidenced, and, if present, they are surely of minor importance. No communications with the blood caverns in the base of the dorsum sellae were found.

Anguis fragilis

Embryo 2,5 mm CR. The main draining vessels of the head at this early stage are represented by the vena capitis medialis of the left and right side. They are located medially to the anlages of the cranial nerve ganglia also posterior to the trigeminal ganglia and it was demonstrated by ink injections from the area vasculosa of the living embryo that these veins are in communication with the heart anlage and thus are to be considered a true drainage of the vascular plexus of the brain anlage. However, no anastomosis between the veins was detected in the pituitary region or elsewhere.

Embryos 33—42 mm TL. The part of the vena capitis medialis behind the ganglion Gasseri is now replaced by the vena capitis lateralis, running distally to the nerve ganglia and the auditory capsule. The venous system in the base of the cranial anlage is similar to that of *Lacerta agilis* of the corresponding stage (5,5 mm CR—28 mm TL). A vena retrohypophysea is formed and constitutes the only outlet for the blood from the vascular plexus of the hypophysial anlage. No capillaries or venules are found running along the epithelial stalk, and it is apparent from the numerous ink-injected embryos of this stage that the thick layer of mesenchyme between the epithelium of the oral roof and the trabeculae and parachordal cartilages does not contain any vascular plexus. Therefore, it seems as if an oral drainage is not developed in this species (cf. p. 229), in spite of the fact that no embryos were available for study in the large gap in the series between the 2,5 mm (CR) embryo and the 33 mm (TL) stage. The vena retrohypophysea is located aborally and attached to the saccus infundibuli, and the anlage of the pars intermedia. It receives a median vein from the plica encephali, exactly as in *Lacerta*. In the bottom of the anlage of the sella ventro-caudally to the hypophysial anlage a congregation of dilated capillaries was found in all investigated specimens (Fig. 11, h). These vessels constitute the forerunners of the vena intrasellaris transversus. Some capillaries from this congregation are seen to follow the internal carotid a short distance caudally. This anlage of the vena intrasellaris always communicates aborally with the vena retrohypophysea via a pair of relatively thin vessels running along the lateral flanks of the hypophysial anlage.

The relation of the vena retrohypophysea to the surrounding structures is similar to the condition in *Lacerta agilis*. The vein is located ventrally to the profundus ganglion and dorsally to the internal carotids. It enters the cranial anlage just in front of the pila antotica together with the retractor bulbi muscles. The latter fill up the main part of the fenestra metoptica and are attached to the trabeculae cranii.

Embryos 46—60 mm TL. Only a few changes in the venous system are noted from the present stage of development. The vena retrohypophysea is a wide vessel (Fig. 32). Exactly as in *Lacerta* an anastomosis between the orbital

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Fig. 32. *Anguis fragilis*. Embryo 46 mm TL. Horizontal section just aborally to the hypophysial anlage showing the size of the vena retrohypophysea. — a = the vena retrohypophysea, b = the vena capitis medialis, c = the internal carotid, d = tuber cinereum, e = the tuberal lobe. — Ink injection, celloidin 60 μ, Mallory's phosphostungstic acid haematoxylin, blue filter, 75 ×.

sinuses is formed, the vena anastomotica. It is located to the rostral margin of the fenestra metoptica as in *Lacerta*. This short anastomosis shows no communication with the hypophysial vascular system. The vena intrasellaris transversus is now a relatively wide-spaced vessel. It has the same communication with the vena retrohypophysea as its anlage in the preceding stage. Some branches following the internal carotids in a caudal direction are always found. It seems as if they are to drain the surroundings of the internal carotids and bring the blood to the vena intrasellaris transversus. No connections between the latter and the vena capitis medialis were found in this species. In a single specimen a median vein enters the sella ventrocaudally and thus connects the growing vascular plexus of the oral roof with the vena intrasellaris.

Embryos 62—73 mm TL. The ossification of the crista sellaris when forming the dorsum sellae, is combined with the establishment of blood caverns. These caverns form a transverse sinuous channel in the base of the dorsum sellae. It communicates with the vascular plexus of the oral roof as well as with the vena intrasellaris transversus. The latter is now a relatively thin vessel in the ventrocaudal corner of the sella, and it also has one or two communications with the rather numerous vessels in the oral roof. However, it has no direct communication with the sinus-system of the adenohypophysis, but its lateral connections with the vena retrohypophysea mentioned in the previous stages still persist. Its branches along the internal carotids are also found. The latter can now be followed as thin but distinct vessels along the carotids caudally to the vicinity of the facialis ganglion where they join the vena capitis lateralis.

As in the preceding stage the vena retrohypophysea seems to be the only outlet of importance for the blood from the hypophysis and its surroundings (Fig. 13). It is a very wide vessel located aborally to the hypophysis close to the tip of the dorsum sellae. Its main tributary is the unpaired median vein from the plica encephali, but it has also several communications with the vascular plexus of the neural lobe and adjacent parts of the adenohypophysis and finally it takes up the two veins from the vena intrasellaris transversus.

Embryos 87—90 mm TL (the hatching stage). This period does not show any changes in the venous system of the pituitary and its surroundings.

The adult stage. The vena retrohypophysea is a very wide channel covering the main part of the dorsal aspect of the hypophysis. Several blood vessels from the neural lobe empty into the vein. There are also some wide communications with the sinus-system of the caudal lobe of the pars distalis. Thus the arrangement of the drainage is similar to that of *Lacerta*. The blood entering the adenohypophysis via the primary plexus and the portal vessels thus at first passes the sinusoids of the cephalic lobe and then the sinusoids of the caudal lobe before reaching the outlet into the vena retrohypophysea.

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The outlet of the vena retrohypophysea is not strictly the same as in *Lacerta*. There is no association with a vena cerebralis media secundaria, but the vein empties directly into the vena capitis medialis. There are also other differences in the venous system of the head compared with *Lacerta*, but they will not be treated here as they do not influence the blood stream from the hypophysis.

The other veins traversing the pituitary region, namely the vena intrasellaris transversus and the caverns included in the bone of the base of the dorsum sellae are relatively tiny and probably play no rôle in the drainage of the hypophysis. It has not been possible to find the communication between the vena intrasellaris transversus and the vena retrohypophysea in the adult *Anguis*, and probably it is not a regular structure as it was in *Lacerta*. The veins which follow the internal carotids and connect the vena intrasellaris and the vena capitis lateralis are found also in the adult.

Natrix natrix

The development of the veins in the head of *Natrix* was studied already by Rathke (1839) dealing of course mainly with the large veins in the outer parts of the head. Grosser and Brezina (1895) made the first detailed study of this system. Grodzinski (1928) re-investigated the development of the vascular system in *Natrix* and he also dealt very thoroughly with the venous system of the head. Descriptions of the said system in the adult grass-snake are given also by Bruner (1907) and, more briefly, by O'Donoghue (1912) and van Gelderen (1924 a). The said descriptions agree in all essentials and have greatly facilitated the solving

of the present problem: to fit the hypophysial drainage into the various ontogenetic venous systems of the developing head. As regards the nomenclature of the veins Grodzinski is mainly followed.

S t a g e I. The blood in the capillaries of the rostral part of the brain anlage is collected by the vena cerebralis anterior. This vein runs backwards over the dorsal margin of the eye anlage and joins the vena orbitalis coming from the region ventral to the eye anlage. This confluence occurs at the level of Rathke's pouch, and the vein is from now on called the vena capitis medialis. It shows the normal course medially to the trigeminal ganglion and then, named vena capitis lateralis, laterally to the auditory-facialis ganglion and the auditory vesicle.

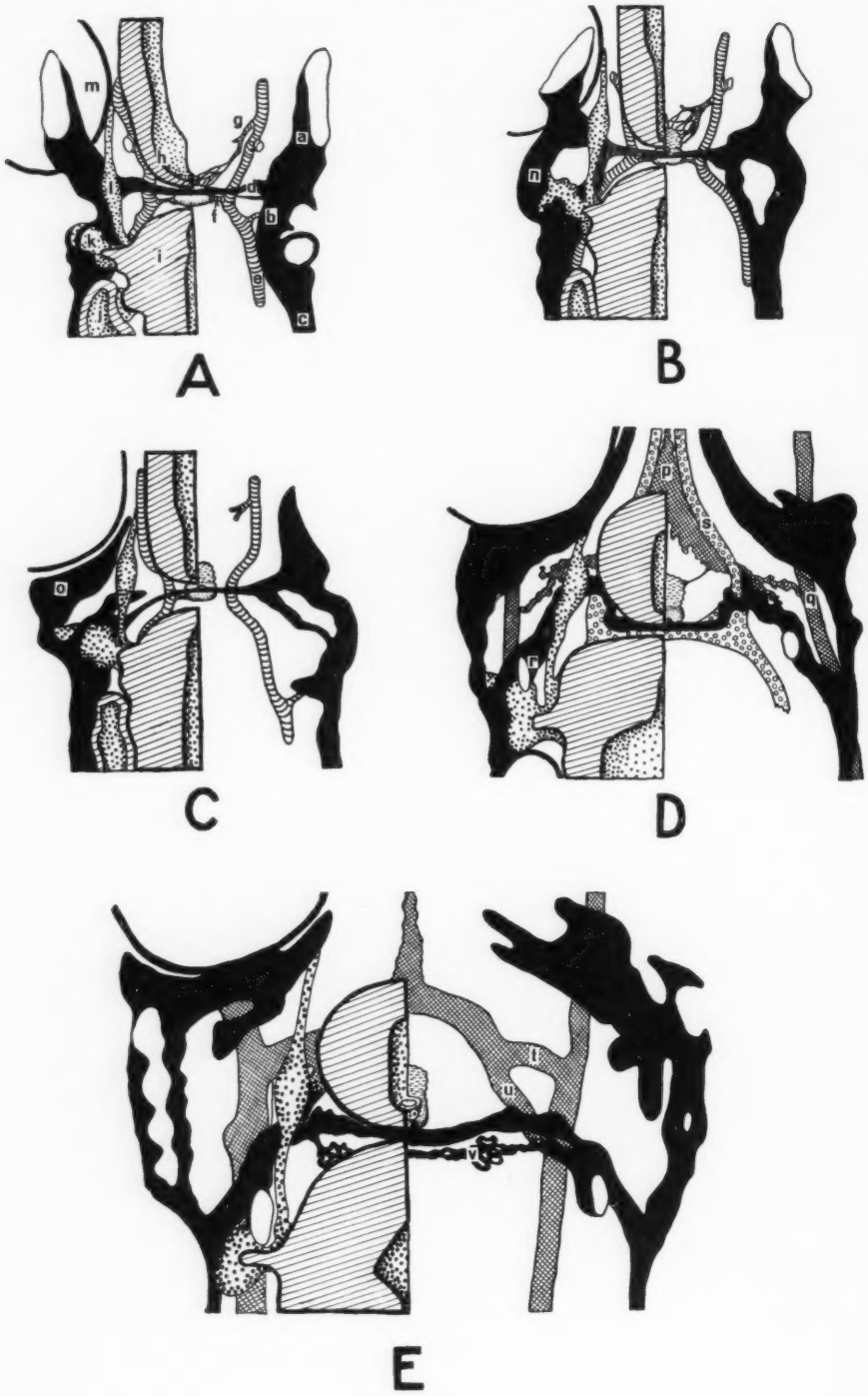
Between the junctions of the vena cerebralis anterior and the vena orbitalis of each side a prominent venous anastomosis, the vena retrohypophysea, is developed (Fig. 33 A). Its outflow in the head veins is located next to the base of the vena cerebralis anterior. The vena retrohypophysea traverses the pituitary region aborally to Rathke's pouch and is there attached to the brain wall. This median part of the vein communicates with the capillaries of the surroundings, i.e. also with the network of the adjacent part of Rathke's pouch, the aboral lobe. It also takes up an unpaired median vein running orally on the anterior brain wall in the plica encephali ventralis. This vein continues to drain the plica up to the adult stage.

Rathke (1839, p. 177) mentions a laterally directed drainage from the pituitary to the "sinus cavernosus" in the third period according to his classification of embryos. It means considerably older embryos than those of stage I in the present investigation. Grosser and Brezina (1895, pp. 308—309) observed a vein running from the pituitary to the vena cerebralis anterior on each side, but in spite of their careful examinations of paraffinsectioned embryos they could not detect the vein until the stage at which the mesenchyme had condensed to form the cartilage anlages of the cranial base. Grodzinski (1928) also mentions probably the same vein from more advanced embryos as "vena hypophysealis". All the cited authors seem to speak about the vein as a hypophysial drainage without mentioning what it really is: a wide anastomosis connecting the venous channels of the left and right side of the head.

The capillaries in the region of the oral contact-zone give rise to distinct veins, one on each side, which empty into the vena orbitalis inferior. Thus the vascular system of the hypophysial anlage has two different draining pathways contrary to what was the case in *Lacerta* and *Anguis*.

S t a g e I I. It has not been possible to detect the veins from the oral contact-zone in all the specimens at this stage. This drainage has surely decreased in importance and is completely reduced later. Thus the drainage via the vena retrohypophysea is the main drainage for the whole hypophysial anlage.

As in the previous stage the vena retrohypophysea is connected to the vena capitis medialis or to the base of the vena cerebralis anterior (Fig. 33 B). As



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pointed out by Grosser and Brezina the vena capitis medialis in *Ophidia* is replaced by another vein running more laterally also in the region of the trigeminal ganglion. Whereas the replacement of the post-trigeminal part of the vena capitis medialis by a vena capitis lateralis seems to be of common occurrence in the *Amniota* this anterior dislocation laterally of the head vein is peculiar to the snakes and a few lizards. This process has begun already in the preceding stage (Fig. 33 B), with the rise of a venous channel connecting the vena capitis lateralis behind the trigeminal ganglion with the vena cerebralis anterior near the orbit. Thus the trigeminal ganglion is surrounded by a venous ring at this intermediate stage.

In more advanced stages the vena capitis medialis is completely reduced (Fig. 33 C). The vena retrohypophysea still has the old communication with the vena cerebralis anterior. The latter vein has now partly formed the sinus orbitalis. However, a newly established vein links the vena retrohypophysea with the vena capitis lateralis in the vicinity of the trigeminal ganglion. This new communication was seen together with the old one in some embryos (Fig. 33 C at the right hand side). The vena retrohypophysea is the most important draining vessel from the hypophysial anlage and no trace of the veins from the oral contact-zone can be found.

Stage III. In the oral roof plexiform sinuses now appear rostrally and orally to the hypophysial anlage. It is the first signs of the sinus palatinus medius (Bruner 1907). Small vessels from the oral part of the vascular system of the hypophysial anlage run orally and join the newly established sinus-system. These vessels are of minor importance in the hypophysial drainage. They are, however, fairly regularly found. They have nothing in common with the veins from the oral contact-zone mentioned earlier.

Fig. 33. *Natrix natrix*. Drawings showing the vena retrohypophysea and its communications with the various ontogenetic venous systems of the basal head up to the adult stage. Dorsal view. A horizontal block comprising the base of the head between the orbit and the auditory capsule is reconstructed. The brain and the nerves are removed to the right of the midline. The head vein system with the vena retrohypophysea is drawn in black. The secondarily formed maxillary vein system with the sinus palatinus is marked with crossed lines. The arteries are drawn as banded vessels. They are left out in D and E. Cut surfaces of the nervous system are obliquely hatched. Large dots indicate the surface of the nerves and of the ventricles. Short horizontal lines denote the oral lobe and fine dots the aboral lobe of the hypophysial anlage. Small circles denote the cartilage of the cranial base. a = vena cerebralis anterior, b = vena capitis medialis, c = vena capitis lateralis, d = vena retrohypophysea, e = internal carotid, f = hypophysial artery, g = infundibular artery, h = diencephalon, i = myelencephalon, j = auditory capsule, k = ganglion Gasseri, l = profundus ganglion, m = eye bulb, n = the most anterior part of the vena capitis lateralis replacing the v. cap. medialis of the pituitary region, o = sinus orbitalis, p = sinus palatinus, q = vena maxillaris, r = vena cerebralis media secundaria, s = trabecula cranii, t = vena palatina obliqua, u = vena palato-cerebralis, v = vena intrasellaris transversus. A = stage I, B = stage I (more advanced), C = stage II, D = stage IV, E = adult. The reconstruction technique is the same as that described under Fig. 30.

Stage IV. The vena retrohypophysea now communicates with the vena capitis lateralis only via the secondary vessel mentioned in the preceding stage (Fig. 33 D). Thus an anastomosis traversing the pituitary region persists between the lateral head veins in spite of the reduction of the vena capitis medialis, and this venous channel is homologous with the original vena retrohypophysea only in its median part. The sinus palatinus medius has increased its extension considerably. It starts between the orbits and extends caudally to the pituitary region where it bifurcates symmetrically, and the resulting branches then run to the secondary continuations of the vena retrohypophysea. At the present stage the vena cerebralis media also empties into the secondary prolongation of the vena retrohypophysea via the vena cerebralis media secundaria according to Grosser and Brezina (as regards the nature and ontogeny of this vessel, compare also Grodzinski). The said plexiform connection between the sinus palatinus medius and the vena cerebralis media secundaria is the forerunner of the vena palato-cerebralis of Bruner (1907). The sinus palatinus medius is also in plexiform connection, the forerunner of the vena palatina obliqua, with the vena maxillaris. The latter vein has grown to a wide channel with increased draining properties in the present stage. Scattered capillaries from the hypophysial anlage to the sinus palatinus medius are always found.

Caudally to the hypophysial anlage below the crista sellaris small sinusoids appear being the first traces of a new transverse venous sinus in the pituitary region.

Stage V. The vena retrohypophysea traverses the hypophysial region aborally to the saccus infundibuli and just in front of the edge of the developing dorsum sellae. It communicates in its median part with the sinus-system of the hypophysial anlage. Caudally to the hypophysis a transverse sinus is formed within the sella. It joins the vena retrohypophysea or the vena palato-cerebralis just outside the sella. This sinus is the vena intrasellaris transversus. Occasionally vascular connections between this vein and the hypophysial vascular system were found at this stage. The vena palato-cerebralis and the vena palatina obliqua are now wide channels. The blood from the hypophysis can now reach the jugular veins via two different path-ways: 1) v. retrohypophysea—v. cerebralis media secundaria (= sinus petrosus superior according to Grodzinski)—v. capitis lateralis, or 2) v. retrohypophysea—v. palato-cerebralis—sinus palatinus medius—v. palatina obliqua—v. maxillaris. This arrangement of the venous system concides with that in the adult snake according to the detailed description by Bruner (1907). (Cf. Fig. 33 E.)

Stage VI. Large separate openings of the vascular systems of the neural lobe and caudal lobe into the vena retrohypophysea were found. The latter vein is located within the connective capsule of the sella. The vena intrasellaris transversus is not engaged in the drainage of the gland. Some vessels from the connective tissue capsule of the hypophysis in one specimen were found to run to this vein.

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The adult stage. The arrangement of the main veins is described above and only a few comments on their relation to the brain case should be added. The vena retrohypophysea collects all blood leaving the hypophysis. It finds its way out from the brain case through a foramen located to the zone where the basisphenoid and the enlarged parietale fuse. Beside the vena retrohypophysea only a median branch of the ramus maxillaris of trigeminus is found in the same foramen, entering the brain case only to leave it again through another narrow foramen in the basisphenoid. Outside the cranial wall the vena retrohypophysea has the said junction with the vena cerebralis media secundaria. The latter takes its exit from the brain case together with the maxillary and mandibular portions of the nervus trigeminus. The remaining main veins mentioned above are all located outside the brain case. It should be noted also that the vena intrasellaris transversus is more or less included in the bone of the basal parts of the dorsum sellae. It has no direct communications with the vascular system of the pituitary gland.

COMPARISONS AND DISCUSSIONS

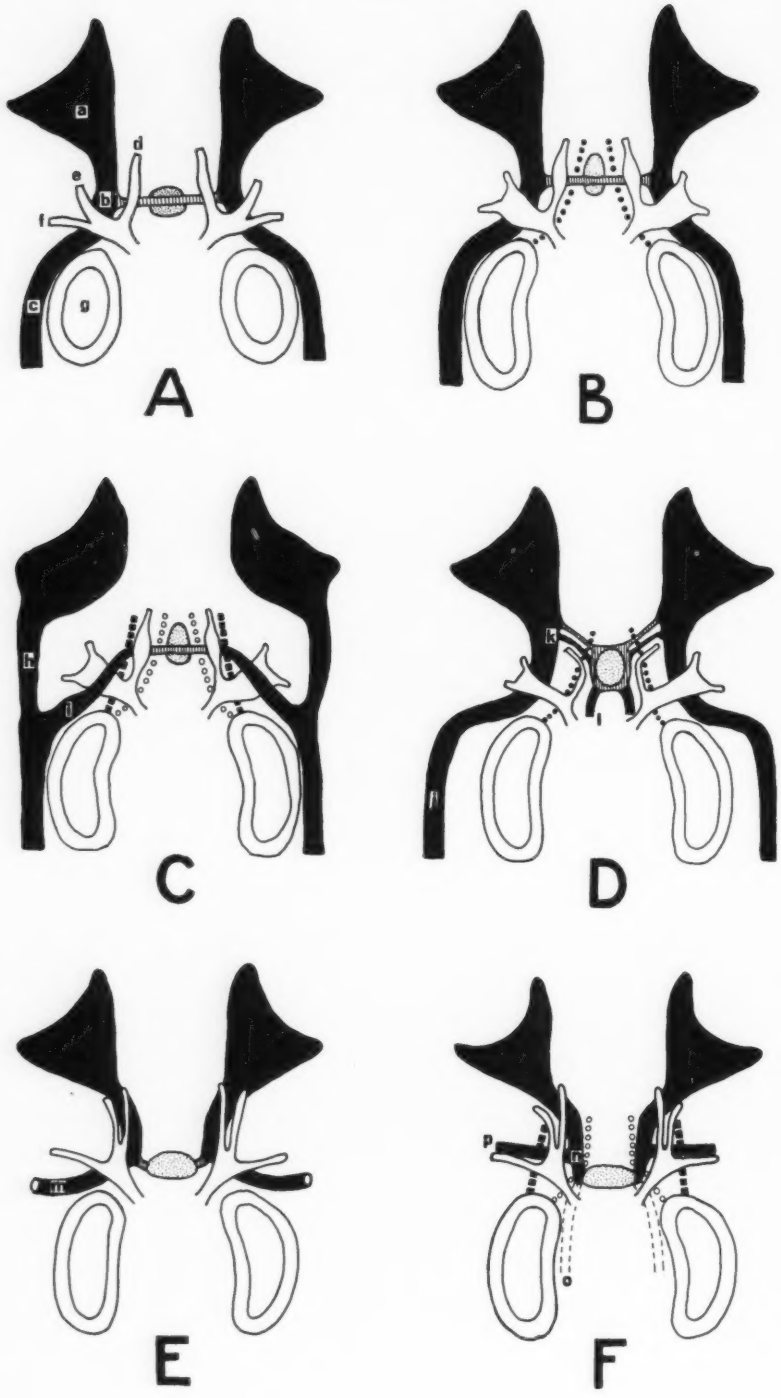
In an earlier paper (Enemar 1957) the venous outlets from the amniotic hypophysis was discussed from a comparative view-point. However, on that occasion nothing was known about the detailed structure of the hypophysial drainage in *Reptilia* (as I had overlooked the paper by Gawrilenko (1930)). The venous anastomosis traversing the hypophysial region in the adult reptile was supposed to be a derivative of a vena retrohypophysea in the embryo and to carry the blood from the hypophysis, and the present investigation was carried out among other things to confirm the true nature of the hypophysial drainage. The comparative discussion will be summarized here and on some points also brought a step further thanks to the experience obtained from the reptilian material. First the development of the definite drainage in the adult amniote—the so-called lateral drainage laid down by the vena retrohypophysea—will be discussed, and secondly other venous outlets—especially the so-called oral drainage—will be dealt with.

The discussion on the structure of the developing cranial wall is based on the comprehensive accounts made by Goodrich (1930) and de Beer (1937).

The lateral drainage.

The development of the lateral drainage in the three amniotic classes is presented in the diagrams in Fig. 34.

As regards the reptiles it has been shown here that the first drainage of the hypophysial anlage is managed by a venous anastomosis traversing the hypophysial region and combining the vena capitis medialis of the left and right side of the head. This anastomosis Shindo (1915) named vena retrohypophysea. He described the vein from embryos of *Lacerta agilis, Chelone viridis,* and *Crocodilus*



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biporcatus. The vein is also taken up as a characteristic structure in the developing cranium by GOODRICH (1930), named the "hypophysial vein", and by DE BEER (1937), with the name "pituitary vein". The vein always enters the cavum cranii through the fenestra metoptica just in front of the pila antotica. After leaving the vena capitis medialis the vena retrohypophysea passes orally to the profundus ganglion and then in the sella aborally to the internal carotid, to the Rathke's pouch and the saccus infundibuli. According to Shindo (op. cit.) the vena retrohypophysea is located ventro-caudally to the hypophysis. This is not true for the three reptiles in the present investigation. Already when the vein first appears in the embryo and throughout the embryonic life and in the adult its position is aborally to the hypophysis. When a ventro-caudal vein was found in the sella it was the secondarily established vena intrasellaris transversus.

The vena retrohypophysea continues to drain the hypophysis without interruption during the embryonic life and in the adult as regards the three investigated reptilian species. In *Lacerta* and *Anguis* there are practically no change in the lateral drainage and its connection with the extra-cranial veins in the adult compared with the embryonic life. The invasion of the retractor bulbi muscles in the sella in *Lacerta agilis* has not prompted any alterations in the venous pathways from the hypophysis (Fig. 33 B).

In this connection attention should be paid to an investigation made by GAWRILENKO (1930). It deals with the developmental processes in the praechordal area in some *Geckonidae,* especially in the species *Hemidactylus frenatus,* and comprises the early period of the development on to the formation of the lateral lobes. Gawrilenko thoroughly describes also the development of the venous arrangement

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Fig. 34. Diagrams showing the hypophysial drainage in the three amniotic classes compared with the anlage of the lateral drainage, the vena retrohypophysea, in the embryo. A = early stage of the embryo, common to the three classes, B = *Lacertilia* (*Lacerta* and *Anguis*) adult, C = *Ophidia* (*Natrix*) adult, D = *Aves* adult, E = *Rodentia* embryo (*Clethrionomys* 14 mm CR), F = *Rodentia* (*Clethrionomys*) adult. Vertically banded vessel = vena retrohypophysea and its derivative, the sinus cavernosus of birds, fine dots = hypophysis, large dots = the original side wall of the neurocranium in the orbito-temporal area, small circles = the original position of the reduced side wall of the neurocranium, black squares = secondarily formed side wall of the cranium (consisting mainly of the parietal bone in *Ophidia,* C, and of the alisphenoid bone in *Mammalia,* F). The cavity between the circles and the squares is the cavum epiptericum. a = sinus orbitalis, b = vena capitis medialis, c = vena capitis lateralis, d, e, and f = the three rami of nervus trigeminus, ramus profundus, maxillaris, and mandibularis respectively, g = auditory capsule, h = the orbito-temporal portion of the vena capitis lateralis in *Ophidia,* i = the secondary communication between the vena retrohypophysea and the vena capitis lateralis in *Ophidia,* j = secondary "Stammvene", k = vena ophthalmica interna (the thin black vessel between the sinus orbitalis and the sinus cavernosus in *Aves,* D), l = vena carotis, m = sinus petrosus superior (v. cerebralis media), n = sinus cavernosus (the mammalian derivative of the vena capitis medialis), o = sinus petrobasilaris (a very small vein in *Clethrionomys* though it constitutes the main drainage of the sinus cavernosus in many other mammals), p = the vein draining the sinus cavernosus, it leaves the cranium together with the mandibular branch of trigeminus.

of the region, although it is not always easy to understand his statements. Gawrilenko found an anastomosis between the venae capitis mediales in the 7 mm embryo of *Hemidactylus* and in a somewhat later stage in *Ptychozoon*. This anastomosis develops into a very heavy venous channel and it is called the "sinus praechordalis". Gawrilenko did not know of Shindo's investigation of 1915, but it is evident that his "sinus praechordalis" exactly corresponds to the vena retrohypophysea. The rise of this vessel is combined with the reduction of the communication between the left and the right praemandibular cavity. It first appears in the midline and secondarily reaches the vena capitis medialis laterally. Gawrilenko also describes the development of an aboral ("anterior") process of the vein. This branch corresponds to the median vein in the plica described in the present investigation. Gawrilenko's drawings based on reconstructions are probably not correct in every respect. Thus the internal carotids are drawn aborally to the "sinus praechordalis" in the figures, for, judging from his figures 3 and 4, he gives the caudal view of the reconstructions. It is surely a mistake, for the sections pictured in figures 14, 15, and 16 in Gawrilenko's paper clearly show that the "sinus praechordalis" is located aborally to the carotids, exactly as is generally valid for the vena retrohypophysea. The communication between this vein and the vessels around Rathke's pouch was also studied by Gawrilenko. Already in the 9,3 mm embryo when the hypophysial anlage is an open pouch he found two veins running aborally along the lateral flanks of the pouch and joining the anastomosis. These small veins were called the "vena hypophyseos dextra et sinistra" and they persisted in the further development as the draining path from the hypophysial vascular system. In older stages the volume of the vena retrohypophysea decreases and the said veins bridge the space between the hypophysis and the vena retrohypophysea which traverses the hypophysial region some distance aborally to the hypophysis. The two venae hypophyseos resemble the veins linking the vena intrasellaris transversus and the vena retrohypophysea in the lizards of the present investigation, but detailed comparisons cannot be made. In *Hemidactylus* the vena capitis medialis is replaced by a vena capitis lateralis also in the orbito-temporal area as in *Natrix,* and Gawrilenko shows how the vena retrohypophysea is linked to the new lateral vein.

Turning to the *Ophidia* it was shown for *Natrix* in the present investigation that the main part of the vena retrohypophysea is retained in the adult, but that its communications with the extra-cranial veins were altered radically during the embryonic life. This process is combined with other differences in the structure of the orbito-temporal area compared with *Lacerta* and *Anguis*. In the last-mentioned two species the original side wall of the neurocranium, in the hypophysial region indicated by the pila antotica and pila metoptica, is maintained in the adult. In *Natrix,* however, as in the *Ophidia* in general, the orbital cartilage and the pillars are reduced, and their function is taken over by the enlarged frontal and parietal bones which extend down to the cranial base and thus give

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rise to a new cranial wall. This process is combined with the inclusion of a new lateral cavity in the brain-case containing among other things the profundus nerve as seen in the diagram (Fig. 34 C). This new cavity corresponds in part to the cavum epiptericum in the *Mammalia* (DE BEER 1937). The vena capitis medialis has escaped the inclusion, for it is early reduced and replaced by a more laterally running vein draining the orbit, as shown in Fig. 33 A—D. In spite of these radical changes, first described by GROSSER and BREZINA (1895), the vena retrohypophysea never shows decreased draining properties, but early receives a new communication with the vena capitis lateralis.

SHINDO (1915) states that the occurrence of a vena retrohypophysea in the embryo is universal in the *Reptilia,* and there is no reason to doubt it. In the investigated members of the *Lacertilia* and *Ophidia* the vein is maintained in the adult as a drainage of the hypophysis and of the plica encephali. Its development into the adult in the *Chelonia* and *Crocodilia* has not yet been investigated.

SÄVE-SÖDERBERGH (1946) gives good information of the veins in the pituitary fossa of *Lacerta vivipara, Varanus salvator,* and *Sphenodon punctatus,* with particular regard to the relation of the veins to the retractor bulbi muscles. In the lacertilian species he describes a vena retrohypophysea (called "vena pituitaria") running dorsally to the retractor musculature and with the same relations to the hypophysis as found in the present investigation. In *Varanus* the vein has a large ventro-caudal extension in the sella. In both species a vena anastomotica was found ventrally to the subiculum infundibuli, as was also the case in *Lacerta agilis* and *Anguis fragilis* (p. 210 and 215).

In *Rhyncocephalia,* however, the arrangement of veins in the surroundings of the hypophysis seems to be partly different. Säve-Söderbergh describes a "vena pituitaria" in *Sphenodon,* but this vein is located ventrocaudally to the hypophysis, a position which resembles that of the vena intrasellaris transversus. It runs ventrally to the retractor muscles, and this circumstance together with different relation to the abducens nerve makes Säve-Söderbergh hesitate as regards the homology between the pituitary veins in the *Lacertilia* and *Rhyncocephalia.* The vena anastomotica is a wide channel in *Sphenodon,* and Säve-Söderbergh could confirm the existence of a left and right vena hypophyseos lateralis, described by O'DONOGHUE (1920). These two veins empty in the vena anastomotica and collect blood when running along the flanks of the pars distalis inside the connective tissue capsule. The "vena pituitaria" is located outside the capsule and there seems to be no doubt that the hypophysis in *Sphenodon* is drained towards the vena anastomotica. The injected specimens of *Lacerta agilis* and *Anguis fragilis* in the author's collection show no communications between the vena anastomotica and the hypophysial vascular system in the embryos or in the adult. This fact together with the universal presence of a vena retrohypophysea in the reptilian embryo and the engagement of the vein in the drainage of the hypophysial anlage indicates that the mode of hypophysial drainage in *Spheno-*

don is not a primitive one. It is reasonable to think that changes in this respect may depend on differences in the process of invasion of the retractor bulbi muscles in the sella. Säve-Söderbergh is of the opinion that this process may probably have gone on independently in different genetic lines. However, our knowledge of the attachment of the retractor bulbi muscles in various reptiles is still very insufficient. Therefore, it seems reasonable to conclude that *Sphenodon* —so primitive in many anatomical structures including the vascular system— has a secondarily modified hypophysial drainage. It has not been possible to obtain any information about the developing vena retrohypophysea from the literature on the embryology of *Sphenodon*. Such information is needed to solve the problem concerning the nature of the "vena pituitaria" in that species.

The establishment and development of the lateral drainage in birds have been described, above all by WINGSTRAND (1951) in *Gallus gallus*, *Larus ridibundus*, and *Riparia riparia*. His statements have been confirmed by GRIGNON (1956) who also used *Gallus*. A vena retrohypophysea is laid down very early during the development, and it is usually called the vena nervi III in birds, as it follows and leaves the cavum cranii together with the oculomotor nerve. The vein is, however, homologous with the vena retrohypophysea in reptiles and mammals as pointed out by WINGSTRAND (op. cit.). Therefore, the latter name will be used also for the birds in the present paper. The vein does not attain the same size as in reptiles, and it is not so strictly located aborally to the top of the Rathke's pouch and to the succeeding stages of the hypophysis as in the reptiles. But it is possible to use the same diagram for the first lateral drainage in birds as for the reptiles (Fig. 34 A) even if the vena retrohypophysea in the early stages of the investigated bird species does not strictly open into the vena capitis medialis, but in the vena cerebralis anterior near the outlet of the latter vein in the vena capitis medialis.

The vena retrohypophysea frequently persists in the adult bird (cf. WINGSTRAND's survey, op. cit. p. 271), but it is usually very thin (Fig. 34 D). It connects the sinus cavernosus which surrounds the pituitary with the sinus orbitalis. The sinus cavernosus is a prominent structure in birds, and it is derived from the median intracranial portion of the vena retrohypophysea. The course of the vein is not so strictly lateral as in reptiles. It runs rostrally for a considerable distance before it leaves the cranial cavity together with the oculomotor nerve (cf. Figs. 154 and 159 in Wingstrand's monograph). This circumstance may be due to divergences in the development in the base of the cranium in the orbito-temporal area in birds compared with the investigated reptiles. For example, a new structure, the pila antotica spuria, is developed behind the true pila antotica, and this pillar separates the profundus nerve from the other trigeminal branches, and therefore the nerve runs intracranially for some distance before leaving the cranium. However, the development of the pila antotica spuria (ossifying to part of the pleurosphenoid in the adult) does not mean the inclusion of any consi-

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derable extracranial cavity into the brain case, and so the function of the vena capitis medialis which drains the sinus orbitalis is not affected. The vein is maintained in the adult, whereas its caudal succession, the vena capitis lateralis, is gradually replaced by a more ventrally running vein, "die sekundäre Stammvene" (VAN GELDEREN 1924 b, HUGHES 1934). The veins exist side by side for a period, and thus the blood stream from the vena capitis medialis is not influenced.

However, secondarily established veins are added to the drainage of the sinus cavernosus in birds. Already in the early embryo but after the formation of the vena retrohypophysea, a new communication between the orbit and the sella is established in the vena ophthalmica interna. This vein is also frequently maintained in the adult bird, but the two pairs of lateral veins from the sinus cavernosus are too narrow to have sufficient draining properties (Fig. 34 D). The drainage is therefore temporarily managed by orally directed veins in a way which will be described later (p. 229). The definite drainage is laid down with the formation of venous pathways along the carotis interna. This vein, the vena carotis, leaves the sella via the foramen caroticum. Wingstrand assumes that the blood flows from the orbit to the sella in the vena retrohypophysea and ophthalmica interna in the adult and that the wide vena carotis alone serves as the outlet from the sinus cavernosus in the sella. Thus the vena retrohypophysea functions as the main outlet from the vascular plexus of the hypophysial anlage only during a very short period of the earliest development (from the 3-day to the 7-day embryo in *Gallus*).

A prominent vena carotis was not found in the investigated reptiles. In some developmental stages only very thin veins were found to run together with the internal carotid, and they were in all cases connected with the vena intrasellaris transversus. They could sometimes be followed to the region of the facialis ganglion where they were seen to join the vena capitis lateralis. Probably small veins regularly follow the carotids, but it is obvious from their minute calibres that they are not engaged in the drainage of the sella to any considerable extent. These veins are present in the adult *Anguis*, but they have no connection with the vascular system of the pituitary. One should remember, also, that SHINDO (1915) has described veins following the carotids out of the sella in *Chelonia*.

Turning to the *Mammalia* it was shown in a previous paper (ENEMAR (1957) that the lateral drainage in the rodent *Clethrionomys glareolus* was laid down with the rise of a vena retrohypophysea in the embryo at the stage when the hypophysial anlage consists of the open Rathke's pouch. The vein is located caudally to the pouch in this species. The same starting diagram as that for reptiles and birds can be used and the following development is shown in the diagrams Figs. 34 E and F, and it will be only briefly discussed here. The vena capitis medialis persists in the adult as the sinus cavernosus, and it is located medially to the trigeminal nerve along the line of the original side wall of the neurocranium in the orbito-temporal area. This cranial wall is, however, re-

duced in the *Mammalia*, and is only represented by a membrane. A new cranial wall is formed laterally by the alisphenoid bone, and the cavity in between is the cavum epiptericum, which contains among others the nervus trigeminus and the sinus cavernosus (vena capitis medialis). The hypophysial anlage grows considerably in lateral direction in the species, and finally, it is practically in contact with the vena capitis medialis. Thus there is no longer any space for the laterally directed veins (the derivatives of the vena retrohypophysea) from the hypophysis. Therefore, the sinus-system of the latter can empty directly in the vena capitis medialis (sinus cavernosus). This secondarily attained contact between the pituitary and the sinus cavernosus in *Mammalia* was interpreted by SHINDO (1915).

In *Clethrionomys* the vena capitis lateralis is reduced and the vena capitis medialis is drained through a dorsolateral vein, the sinus petrosus superior (Fig. 34 E). Then also the latter becomes vestigial, and the vena capitis medialis (sinus cavernosus) is drained via a new vein which takes its exit from the cavum epiptericum together with the mandibular branch of the trigeminal nerve (Fig. 34 F).

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The question is whether the development of the lateral drainage in *Clethrionomys* is generally valid for *Mammalia*. It is obvious that the fate of the vena capitis medialis is the same generally speaking. Its final outlets in the adult varies apparently from one species to another (cf. STREETER 1915, SHINDO 1915, VAN GELDEREN 1924 and others). In some species, e.g. *Acrobates* and *Didelphys* (SHINDO), *Canis* (DENNSTEDT 1903), and *Monotremata* (HOCHSTETTER 1896, HANSTRÖM and WINGSTRAND 1951) a vena carotis drains the sella. This variation does not exclude the existence of a vena retrohypophysea in the embryo. However, information of the existence of the vein in the mammalian embryo is scarce in the literature. SHINDO (op. cit.) describes the vein from *Mus* and from *Lepus cuniculus*. In papers on the development of the hypophysial vascular system the drainage is usually only lightly touched. In *Homo* there are veins from the developing pars distalis running laterally. They are seen on the microphotographs in Wislocki's paper. Such veins are also seen in the reconstructions made by BOERNER-PATZELT (1954). They are also found in embryos of the laboratory rat according to MIRAGLIA (1958). The said lateral veins probably exist in all mammalian embryos in the stages before the hypophysis and the sinus cavernosus meet. It is reasonable to think that they are also derived from the vena retrohypophysea in the early embryo as in *Clethrionomys*.

Summing up, the results obtained from the reptilian embryos in the present investigation as regards the structure and development of the hypophysial drainage underline the conclusion drawn in the author's paper of 1957 that the lateral drainage of the hypophysis in the *Amniota* is primitive from a phyletic point of view. It is laid down in the early embryo by the formation of the vena retrohypophysea in all classes. This vein is retained as a draining path for the hypophysis in adult reptiles (at least in *Lacertilia* and *Ophidia*, though exceptions

are found, cf. *Rhyncocephalia*) whereas it is reduced as a draining vein from the gland in the adult birds and mammals.

The oral drainage.

WINGSTRAND (1951) found that the vascular plexus of the hypophysial anlage in birds was temporarily drained by veins leaving the sella turcica through the fenestra hypophyseos in an oral direction. This oral drainage replaced the vena retrohypophysea when the latter vein had become vestigial, and the oral drainage then functions until the fenestra is closed. At this time the permanent drainage of the sella via the vena carotis already functions. The described development was found in the embryos of *Larus* and *Gallus*, whereas in *Riparia* the oral drainage seemed to be eliminated. GRIGNON (1956) also found a prominent oral drainage in *Gallus*.

In mammals an oral drainage is described only from embryos of *Clethrionomys* and of *Apodemus* (ENEMAR 1957). Other papers on the development of the hypophysial vascular system have not paid special attention to the drainage. However, GLYDON (1957) observed capillaries in the stroma of the epithelial stalk in the embryo fifteen days after insemination in the albino rat. Such vessels persisted in the eighteen day stage. Glydon considers that these vessels supplied the hypophysial plexus, but he was surely mistaken owing to the fact that he did not study the venous outlets. Glydon's study was concentrated on the development of the hypophysial portal system. Therefore, his observations of the vessels following the epithelial stalk denote that an oral drainage is present also in the embryo of the albino rat.

In my previous study (1957) I supposed that the oral drainage is an ontogenetic phenomenon without phylogenetic significance. The information obtained from the reptilian material in the present investigation supports this view. In *Lacerta* and *Anguis* it was not possible to detect any vessels running along the epithelial stalk of the extra-cranial veins. The veins running from the vena intrasellaris transversus to vena capitis medialis in *Lacerta* (p. 211) never act as an oral drainage of the hypophysial anlage. They are developed when the epithelial stalk has obliterated, i.e. comparatively late during the embryonic life. In *Natrix* separate thin veins are seen to leave the vascular plexus of the oral contact-zone and join the anlage of the sinus orbitalis in the earliest investigated stage (p. 217). These veins were not associated with the epithelial stalk, and they soon disappeared. They seem to be extremely short-lived compared with the oral drainage in birds and rodents. In the last-mentioned groups the oral drainage ceases to exist when the foramen cranio-pharyngeus is closed, in *Natrix* much earlier.

In reptiles the vena retrohypophysea is a prominent vessel from the beginning, and it never shows any reduction and therefore it can function as a sufficient draining pathway from the hypophysis continuously. So to speak, an oral

drainage is never needed in the investigated species. As pointed out earlier (p. 175) the hypophysial anlage in *Natrix* is very long in a rostro-caudal direction, and in stage I it also has a double supply. The communication with the vena retrohypophysea may be insufficiently developed at this stage, especially between the vein and the rich capillary congregation surrounding the oral contact-zone far away. The aboral lobe of the hypophysial anlage is supplied by the very wide hypophysial arteries in that stage and it seems reasonable that this circumstance has prompted the rise of a temporarily special drainage for the blood from the oral contact-zone to the anlage of the sinus orbitalis (vena orbitalis inferior) nearby. This drainage is not comparable with the oral drainage in birds and mammals.

In birds the oral drainage is easily explained as being a coenogenetic structure, because it is the only drainage of importance when the vena retrohypophysea is reduced to a minute vessel and before the vena carotis grows to a vein with sufficient draining properties.

As far as the mammals are concerned too few species have hitherto been studied as to the nature of the oral drainage to draw general conclusions. In *Clethrionomys* the oral drainage is prominent, but it always functions side by side with the lateral drainage. It is the main outlet in the 8—9 mm embryo. At that particular stage the vena retrohypophysea is stretched considerably owing to the caudal dislocation of the hypophysial anlage, and it also appears rather thin and probably has diminished draining properties. The presence of an oral drainage seems to be necessary to maintain the blood stream from the hypophysis. This oral drainage at least in part covers the critical period in the lateral drainage caused by the dislocation of the hypophysial anlage in relation to the surrounding main vessels.

To sum up, the most reasonable interpretation of the oral drainage in the *Amniota* is to ascribe to it a coenogenetic nature. This view is supported by the fact that the oral drainage appears during the development only in the avian and mammalian embryos hitherto investigated, i.e. the members of the amniotic classes in which the primary draining vessel, the vena retrohypophysea, is reduced or strongly modified during the embryonic life.

Notes on the sinus cavernosus and the vena retrohypophysea.

A so-called sinus cavernosus is found in the vicinity of the hypophysis in birds and mammals. In the descriptions of the hypophysial vascular systems it is often said that the system opens into the sinus cavernosus. This structure is, however, very variable as to its size and form and further, it is not homologous throughout in birds and mammals.

In the reptiles of the present investigation a sinus cavernosus is completely lacking. Shindo (1915) made an extensive study on the sinus cavernosus in

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Mammalia and its corresponding structure in *Reptilia*, and he found that the mammalian sinus cavernosus is homologous with part of the vena capitis medialis in the *Reptilia*. As shown here both the structures receive the blood from the hypophysis with the exception of the species in which the vena capitis medialis is reduced also in the orbito-temporal area (snakes, a few lizards).

Shindo in the *Mammalia* distinguished the lateral portions of the sinus cavernosus being the derivative of the vena capitis medialis from the sinus intercavernosus anterior (nasalis) and the sinus intercavernosus posterior (caudalis). The said intercavernous sinuses are communications between the left and right sinus cavernosus in front of and behind the hypophysis. According to Shindo the sinus intercavernosus posterior is derived from the vena retrohypophysea. This sinus is not by far always developed. In *Clethrionomys* (Enemar 1957) no sinus intercavernosus is found although a vena retrohypophysea is formed during the embryonic life. Cavernous sinuses show on the whole a poor development in the rodents. It should also be remembered that the development of the hypophysial drainage has not yet been followed in a mammal in which a sinus intercavernosus posterior is formed. Perhaps the connection between the anlage of the hypophysial drainage and the vena retrohypophysea will appear still more clearly in such a species than in *Clethrionomys*.

In birds the sinus cavernosus is a conspicuous structure in the sella, but it is not homologous with the whole sinus cavernosus in the *Mammalia* as pointed out by Wingstrand (1951). The avian sinus cavernosus is derived from the vena retrohypophysea only and thus corresponds only to the sinus intercavernosus posterior in the *Mammalia*. The homologon to the mammalian sinus cavernosus in the birds is not included in the braincase to the neighbourhood of the pituitary, but remains as the extracranial vena capitis medialis. Consequently, the avian sinus cavernosus in Fig. 34 D is marked out in the same way as the vena retrohypophysea.

Finally, some remarks may be added to the characteristic of the vena retrohypophysea. This vein is still insufficiently known in the *Amniota*, but it shows some attributes common to the three classes:

1. It is laid down as an anastomosis between the venae capitis mediales in the orbito-temporal area.

2. It is developed in front of the pila antotica and dorsally to the internal carotids (cf. also Goodrich 1930).

3. It is early engaged in the drainage of the vascular system of the hypophysial anlage.

The localization of the vena retrohypophysea in relation to Rathke's pouch shows some variations. In the reptiles of the present investigation it traverses the pituitary region aborally to Rathke's pouch. In birds (Wingstrand 1951) and in *Clethrionomys* (Enemar 1957) it is situated near the caudal wall of the pouch, a position which is described also by Shindo (1915) in reptiles and mammals.

In the reptiles the vena retrohypophysea is also engaged in the drainage of the plica encephali. An unpaired median vein enters the vena retrohypophysea from above and is laid down in the investigated reptilian embryos very early, and it is then preserved as a functioning vein also in the adult stage. This vein is not described from birds and mammals, and it is sometimes reduced in reptiles (*Hemidactylus* according to GAWRILENKO 1930). The question is whether the engagement of the vena retrohypophysea in the drainage of the plica is a phylogenetically old arrangement as in the case of the hypophysial drainage. In *Clethrionomys* the vena retrohypophysea has two heavy branches which drain the brain wall aborally to the saccus infundibuli (see ENEMAR 1957, Fig. 14). This drainage is, however, very early reduced, and in the succeding stages and in the adult the plica is drained to other veins of the head.

Summary

The development of the hypophysial vascular system is described in detail for *Lacerta agilis*, *Anguis fragilis*, and *Natrix natrix*. The hypophysial portal system in the adult was found to agree with the structure of the corresponding vascular system of *Aves* and *Mammalia*, and it is also laid down and differentiated mainly according to the same basic pattern. A closer examination revealed some differences between *Reptilia* and *Mammalia* which can be explained by the fact that the most anterior part (the cephalic lobe) of the reptilian pars distalis is almost completely reduced in *Mammalia*. In that way the study of the developing hypophysial vessels supports the homologies of the amniotic hypophysis as they have been worked out by Woerdeman and by Wingstrand. Some structures in the mammalian adenohypophysial anlage are discussed and interpreted as corresponding to the anlage of the cephalic lobe of the reptilian pars distalis.

In *Lacerta* and *Anguis* the adenohypophysial anlage is initially supplied only via capillaries from the adjacent brain wall. These so-called linking capillaries are later on transformed into portal vessels. In *Natrix* the early anlage has a strong direct arterial supply, but thereafter the supply via the linking capillaries gradually increases in importance and finally these capillaries furnish the main blood stream to the anlage.

The development of the primary plexus of the hypophysial portal system could be followed fairly exactly in *Lacerta*. The lateral lobes (the anlage of the pars tuberalis) show no relation to the developing portal vessels or to the primary plexus in *Lacerta* and *Anguis*. In *Natrix* the lateral lobes are vestigial.

The ontogeny of the draining path-ways from the hypophysis was studied in the three species. The first drainage of the hypophysial anlage is laid down with the formation of the vena retrohypophysea, and this vein is retained as the definite drainage in the adult. No typical temporary drainage along the epithelial

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stalk of the hypophysial anlage, the so-called oral drainage, was found. The development of the hypophysial drainage in the three amniotic classes is compared, and the investigated reptiles are found to have the most primitive hypophysial drainage from a phyletic point of view.

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Specific Silver Staining of Nerve Fibres

1. Technique for Vertebrates

by

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Introduction

Solutions of silver compounds were originally employed for staining of epithelial and connective tissue in *fresh* material by v. RECKLINGHAUSEN (1860) among others. RANVIER (1872) found that silver nitrate had affinity also to nerves. The staining was effected by the reduction power of the *sunlight*.

However, not until BIELSCHOWSKY (1902) and CAJAL (1903) started to stain *fixed* material and to develop the silver staining by chemicals could the nervous system be more properly demonstrated.

BIELSCHOWSKY used ammoniacal silver solutions (diammine-silver ions) for material fixed in formalin and pre-treated with a solution of silver nitrate. Formalin was also used as a developer.

Cajal, on the other hand, employed alcoholic fixatives and obtained block staining by impregnation in a silver nitrate solution and subsequent development with hydroquinone or pyrogallol.

Since then innumerable more or less valuable variants have been proposed owing to unsatisfactory results or, in many cases, even failure.

A particularly useful modification of the Bielschowsky technique was introduced by SCHULTZE—GROS (1918). It is adapted for frozen sections and thus unsuitable in all those cases where frozen sections cannot be used. Besides, the staining is coarse-fibred and unsatisfactory from a histological view-point.

Therefore, when BODIAN (1936) discovered the favourable effect of the addition of metallic copper to a solution of protargol, which had already been successfully employed by WALTER (1913), the protargol technique was generally accepted as a method of unusual merit. It is adapted for mounted paraffin sections, and very useful from a histological point of view. The main drawback is the unknown chemical composition and the varying quality of different protargol preparations. Unfortunately the excellent pre-war protargol vanished from the market and later preparations have not been equally useful.

These facts gave rise to an intensive search for substitutional methods. Thus, HOLMES (1943) impregnated sections with very much diluted solutions of silver nitrate (1 : 10.000—1 : 30.000) in a borax-boric acid buffer solution at about pH 8,0, and obtained results, which were similar to those produced by protargol without copper. The pH was not decreased as in a protargol solution with copper.

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DAVENPORT, MCARTHUR, and BRUESCH (1939) stated that a pre-treatment with a strong and, hence, acid silver nitrate solution improved the staining obtained with protargol. In accordance with this, HOLMES (1947) started with a strong silver nitrate solution and continued with the diluted, buffered solution, to which also some pyridine (dipyridine-silver ions) was added. For many purposes this is a very useful method.

The mechanism of HOLMES's *initial* method (1943) was studied and the technique modified by ROMANES (1950), SAMUEL (1953), and PETERS (1955 a—e). SAMUEL and PETERS removed the reducible silver from the impregnated sections with a solution of sodium sulphite, and obtained staining by a subsequent physical development (i.e. action of a developer containing and producing nascent silver). The practical results were scarcely equal to those obtainable with HOLMES's *modified* method (1947) but very meritoriously these authors drew the attention to *the importance of a controlled staining procedure.*

Meanwhile many attempts were made to substitute organic compounds such as peptones or other split protein products (PORTER and DAVENPORT, 1951), amino-acids (BROWN and VOGELAAR, 1956), or eggalbumen (PETERS, 1958) for the organic components of the protargol.

In spite of the very great number of methods now available for silver staining of nerve fibres new methods or variants are frequently proposed. Evidently the authors have often erroneously concluded that a variant found to be useful for the material in hand may also be useful for other ones. However, no existing method is in fact able to stain the nerve fibres in all specimens. The stainability differs within wide limits, and each method has its own limited range of utility.

As regards the invertebrates, for instance, the results are generally very unsatisfactory or the failure is complete.

The author studied the reason for this and tried to find a technique for the adaptation of the silver staining to the different stainability of the nervous system throughout the whole animal kingdom. The technique has been successfully tested for a great many species, though, naturally, not for all those existing. Thus, further experience will show to what extent this aim has been achieved.

In the following pages the mechanism of the method will be discussed and a description given for the silver staining of nerve fibres in vertebrates. It is the author's intention to deal with the silver staining of the nervous system of invertebrates in a second part of this communication.

The Silver Impregnation

Every silver staining procedure is started with treatment (impregnation) in a solution of a silver compound. The silver ions combine reversibly with the tissue substances, and are partly reduced to so-called 'silver nuclei' (Liesegang, 1911). Probably silver ions are also electrostatically adsorbed to the silver nuclei (and other particles) (Palmgren, 1948).

Almost every tissue element is silver stainable (though not necessarily specifically) and thus able to combine with and reduce silver ions. However, the very possibility of obtaining *specific* stainings as well as the fact that the stainability varies premises differences in the chemical composition and probably also in the physical state (Lipp, 1951) of different tissue elements. Theories on the nature of some functional groups, which react more readily with the silver ions than others, have been discussed by Romanes (1950), Wolman (1955) and Peters (1955 a).

From a purely practical view-point it is an important fact that the tissue proteins react with formaldehyde. Formalin used either as a fixative or for pretreatment of sections from material fixed in alcoholic fixatives decidedly influences the staining.

Factors influencing the impregnation are 1) the concentration of silver ions, 2) the pH, 3) the time, and 4) the temperature (Davenport, McArthur, and Bruesch, 1939).

Within limits these factors are inversely proportional to each other. Thus, for instance, when the concentration of silver ions is low, the pH must be high and *vice versa.*

Like the silver ions the hydrogen ions combine reversibly with the tissue substances in such a way that the silver and the hydrogen (and some other) ions could be regarded as competitors.

Specific silver staining of nerve fibres has been reported by different authors after impregnation with concentrations of silver nitrate ranging from 40 % to

1 : 30.000, and within a range pH 5,0—9,0. Furthermore, successful stainings have been obtained after impregnation at temperatures between room-temperature and 56° C (or even at still higher temperatures). The time-factor has been varied (for sections) from 2 minutes to 48 hours.

Hence follows, that the *absolute* values of the four interrelative factors are unimportant within rather wide limits. The *relative* values, on the other hand, are absolutely essential. Thus, when three of the four factors are kept constant and the fourth varied, the last-mentioned factor appears to be the determining one, generally within very narrow limits, not because of its absolute but relative value, however.

This is a general rule. All factors influencing the complete staining procedure are actually correlative. Therefore, general conclusions as to the mechanism of the silver staining should not be drawn on the basis of experiments where the influence of some, but not all, factors have been studied. In such cases the absolute value of the varied factors is easily and erroneously considered to be particularly important. Thus, for instance, the silver staining of nerve fibres does not *require* a high pH-level. The reason why a high pH-level is generally used and considered to be necessary is that the concentration of free silver ions is very low in protargol and other commonly used solutions. In fact, equally useful, or even better, results are obtainable with a high concentration of silver ions at a low pH-level.

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With these considerations in mind the composition of the impregnation fluid as well as the time and the temperature could evidently be rather freely selected, mainly with regard to practical advantages and drawbacks.

Because of the importance of a controlled staining procedure, silver nitrate is the most suitable compound. It can always be obtained as a chemically pure and well-defined substance. Besides, it is more stable than most other soluble silver compounds.

The Concentration of Silver Ions, and the pH.—For the present method a high concentration, 10 %, is preferable because, within limits, the amount of combined silver ions in the section is proportional to the concentration of the free silver ions in the solution (Peters, 1955 a). In a strong solution of silver nitrate a suitable low pH is automatically obtained by hydrolysis. Small but sometimes very important adjustments of the pH-level can easily be made by dissolving the silver nitrate in very much diluted solutions of either nitric acid (N/1000) or pyridine (0,02 %) instead of in distilled water. Thus, in the present method the pH is used as a variable in the form of three different impregnation fluids, namely (1) 10 % $AgNO_3$ in N/1000 nitric acid (the impregnation fluid referred to in the following as I_1), (2) 10 % $AgNO_3$ in distilled water (the fluid I_2), and (3) 10 % $AgNO_3$ in 0,02 % aqueous solution of pyridine (the fluid I_3).

The Time.—The amount of reduced silver (the number of silver nuclei) which forms in the section during the impregnation increases at first rapidly, later more

slowly, and at last the reduction practically ceases (Lipp, 1951). Also the amount of combined silver ions reaches a maximum in a similar way (Romanes, 1950).

It is highly probable that the combination of the silver ions with the argyrophilic tissue substances is a prerequisite for the reduction. Both these processes reach a maximum or an equilibrium, which is dependent on the concentration of the free silver ions and the competitory hydrogen ions in the solution.

For the silver staining of nerve fibres the suitable time of impregnation (for the present method) is very often much shorter than the maximum time. Generally the impregnation type I_1 requires 16—24 hours (overnight), while about 10—15 minutes is sufficient when the impregnation type I_2 or I_3 is used for material fixed in fixatives which contain formalin or pre-treated with formalin.

Detailed directions for the use of the two variables, namely the pH, and the impregnation time will be given in the following. Of course a rigid method without variables is more convenient, but probably the range of utility of such a method will never be wide enough.

The Temperature.—Reliably uniform results require a controlled temperature, which is most easily obtained in an incubator. Generally, 37° C is used and also employed in the present method.

The Result of the Impregnation.—The *silver nuclei* are invisible and not detectable even with the electron microscope (Peters, 1955 d). However, where they are sufficiently abundant they can be indirectly visualized by the following method.

(1) Deparaffinize and take to distilled water as usual, (2) impregnate with 10 % aq. $AgNO_3$ for 24 hours at 37° C, (3) rinse through 5 changes of distilled water for about 2 minutes in all, (4) treat in 2 % aq. Na_2SO_3 for 5 minutes (to remove the combined silver ions, Samuel, 1953), (5) rinse through 3 changes of distilled water for 5—10 minutes, (6) tone with the gold chloride solution used in the present method, rinse briefly in water, and intensify as in this method, including the additional intensifying described in the following, (7) complete the procedure by rinsing, dehydration and mounting.—It should be noticed that the development (reduction of silver ions by chemicals) is omitted.

Also the distribution of the *combined silver ions* could be indirectly determined as follows.

(1) Deparaffinize, transfer to absolute alcohol, and coat the sections with a thin layer of collodion (½ % celloidin in alcohol-ether). Take to distilled water as usual, (2) impregnate in 10 % aq. $AgNO_3$ for 24 hours at 37° C., (3) rinse through 5 changes of distilled water for about 2 minutes in all, (4) precipitate the silver ions by treatment in 1 % aq. potassium bromide for about 1 minute, and rinse in distilled water, (5) remove the silver nuclei by treatment in a saturated solution of nitric acid for 10—15 seconds, agitating the slide all the time, and transfer at once to 95 % alcohol for a few minutes, (6) rinse in distilled water, (7) develop in a fresh-made solution of 1 g. hydroquinone and 0,5 g.

Fig. 1. The distribution of the *silver nuclei* in blood corpuscles and striated muscle fibres after impregnation in 10 % silver nitrate solution for 24 hours at 37° C., determined by the method described in the text. Part of a transverse section of hag-fish, fixed in Bodian's fluid, formula 2, from the same series as in Fig. 2. 500 ×.

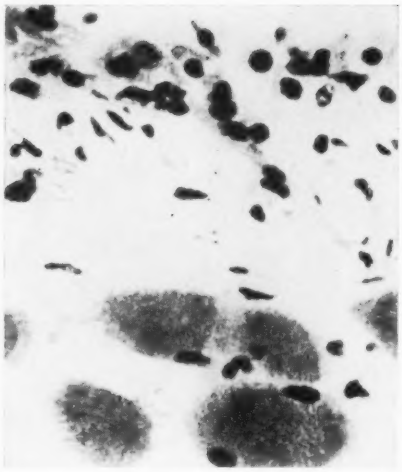

Fig. 2. The distribution of the *combined silver ions* in blood corpuscles and striated muscle fibres after impregnation in 10 % silver nitrate solution for 24 hours at 37° C., determined by the method described in the text. Transverse section of hag-fish from the same series as in Fig. 1. 500 ×.

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borax in 100 ml. distilled water for 15 minutes at room-temperature, (8) tone and intensify as in the present method, rinse, fix, dehydrate and cover.—It should be noticed that all the reduced silver is momentarily dissolved in the saturated nitric acid, whereas the silver bromide is insoluble in this acid.

These two methods were used for consecutive sections in a series of sections from *Myxine glutinosa,* fixed in Bodian's fluid, formula 2 (1937), among other series.

After an impregnation in 10 % silver nitrate solution for 24 hours at 37° C the *silver nuclei* were particularly abundant in the cyto—and sarcoplasm. (Fig. 1). Neither cell nuclei nor nerve fibres contained any accumulation of silver nuclei.

The *combined silver ions,* on the other hand, were preferably found in the cell nuclei, which stained black (Fig. 2), and also in the nerve fibres, which stained purplish with *this* method.

With the *present* method the cell nuclei and the nerve fibres, which contain comparatively large amounts of combined silver ions but only few silver nuclei, stain intensely black. A purplish staining is always obtained where the silver nuclei are particularly concentrated (provided that the rate of the development is high).

However, the impregnation *alone* involves no useful staining of any kind. In

fact, the very best final results are obtained when the sections remain almost unstained during the impregnation, that is to say when the amount of reduced silver is small and consequently the amount of unreduced silver comparatively large.

The Development

Silver ions and silver nuclei are the two main prerequisites to a final silver staining. Silver nuclei serve as acceptors of additional silver, deposited by the reduction of silver ions with a developing agent.

As a developer Schultze—Gros (1918) used formalin, liberated from the formalin-fixed tissues, and acting upon diammine-silver ions (and thus very small amounts of free silver ions) at an extremely high pH-level (about 12,3, Lipp, 1951).

Bodian (1936) and Holmes (1943) reduced the combined and the remaining free silver ions within the sections with a hydroquinone-sodium sulphite solution at about pH 8,5.

Peters (1955 e) removed the silver ions from the sections (with sodium sulphite) and developed with a physical developer at pH 6,0—6,5.

Palmgren (1948, 1955) converted a large surplus of free silver ions in the sections into nascent silver with an acidified, alcoholic pyrogallol solution at pH 4,0.

Thus, not even at the development the staining of the nerve fibres requires any definite pH-level which is independent of other factors influencing the staining process. When the concentration of silver ions is low, the pH should be high and *vice versa,* quite as during the impregnation.

In the present method the *free* silver ions are completely removed by rinsing previous to the development. The concentration of the remaining combined silver ions is low. Consequently the pH should be high during the development.

The silver staining obtained with the present method is only partly visible and must always (as in Bodian's and Holmes's methods) be toned and intensified. Thus, the final staining is not a silver but a gold staining.

The final gold staining is or should be either black or purplish. The tinge was studied electron-microscopically. In a series of cross-sections at 15 and at 50 microns from an earthworm, fixed in Bodian's fluid, formula 2 (1937), the intestinal nerve plexus was stained with the present method. After impregnation for 30 minutes in the impregnation fluid I_1 at 37° C, rinsing, development at the highest rate (see below), toning, intensifying and so on, the nerve fibres stained intensely black and the non-nervous tissues light purplish (Fig. 3). When the impregnation was prolonged to 24 hours the nerve fibres showed a purplish-black tinge and the surrounding tissues a more pronounced purplish staining. The thick

Fig. 3. Part of the intestinal nerve plexus of an earthworm, fixed in Bodian's fluid, formula 2. Stained by the formula I_1 *30 minutes* D_1 (see the text). Arrow points to the dense part of the plexus shown in Fig. 4. 500 ×.

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sections were stained, then embedded and re-sectioned with an ultra-microtome, and studied with the electron-microscope (Siemens Elmiskop I).

As could be concluded from the well-known fact that the colour of a colloidal gold suspension changes from red to black when the size of the particles increases, the black staining consists of comparatively very large, spherical granules and the purplish of scattered, few and very small particles (Figs. 4 and 5). After the prolonged impregnation the number of deposition centres was increased (Fig. 6 and 7), and in the nerve fibres the granules became smaller, probably because the limited amount of nascent silver was dispersed over such a great number of deposition centres that each particle had to be small (Compare Fig. 5 and 6). Evidently the number of deposition centres is an important factor.

The following experiments gave further evidence of the mechanism of the present method. Among other sections a series of cross-sections from *Myxine glutinosa,* fixed in Bodian's fluid, formula 2, was used. After the development the sections were toned and the gold staining intensified as in the present method. Thus, the description of the result refers to the final gold staining.

Exp. 1. Impregnation in 10 % aq. $AgNO_3$ *for 15 minutes* at 37° C. Then rinsing through 5 changes of distilled water (pH 6,2) for 2 minutes in all (at 20° C) and development in 1 % aq. hydroquione (pH 5,7) for 10 minutes at 20° C. Finally rinsing, toning, intensifying, and so on.

Owing to the comparatively slow development in a plain hydroquinone solution at a low pH-level and the small number of silver nuclei obtained during an impregnation for 15 minutes only, *the acceptor mechanism was ineffective.* The rather poor 'staining' consisted of scattered, minute black granules only (Fig. 8). This is the typical result of an 'underimpregnation'.

Fig. 4. The fine structure of the *black* stained intestinal nerve plexus of an earthworm. Section from the same series as in Fig. 3, stained in the same way, then resectioned and studied with electron microscope. Arrows point to minute scattered granules constituting the *light purplish* counter-staining. 15.000 ×.

Exp. 2. Treatments as in exp. 1 with the only exception that *the impregnation time* was prolonged to 24 hours.

For the given mode of development the nerve fibres still contained too few

Fig. 5. Detail of the same nerve plexus as in Fig. 4. 160.000 ×.

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Fig. 6. Same nerve plexus as in Fig. 3 and 4, but stained by the formula I_1 *24 hours* D_1 (see the text). The *purplish black* nerve fibres contain more numerous but smaller particles than the black nerve fibres in Fig. 5. 160.000 ×.

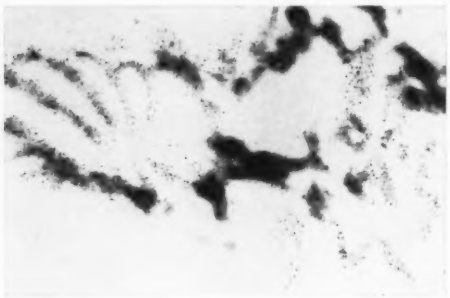

Fig. 7. Numerous scattered particles constituting the *dark purplish* counter-staining in the section Fig. 6. Compare at arrows Fig. 4. 15.000 ×.

silver nuclei and remained unstained, while other tissues stained completely. The mucous cells, the blood corpuscles, and the connective tissue stained purplish, the striated muscle purplish or greyish, and the cell nuclei black.

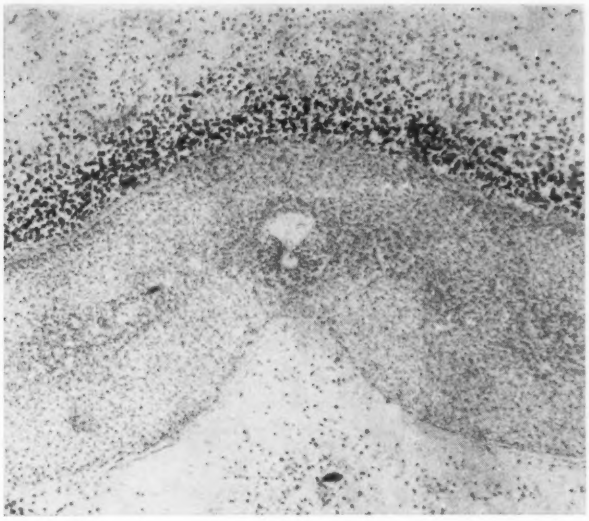

Fig. 8. Part of the spinal cord of hag-fish, fixed in Bodian's fluid, formula 2. The result of experiment 1 (see the text). The typical appearance of a 'staining' after an insufficient impregnation. 150 ×.

Evidently the ability to reduce silver ions is *less* pronounced in the axons than at other sites.

Exp. 3. Treatments as in exp. 1 with the only exception that the pH-level in the developer was elevated (to 8,9) by the addition of *0,5 g. borax to 100 ml. of the hydroquinone solution.* This alkaline solution is unstable and was fresh-made. Of course, at this high pH-level the rate of development was much increased, and the silver ions mainly deposited *in situ.*

The staining was complete. The cell nuclei stained black or purplish-black most other tissue elements purplish, and the nerve fibres greyish or black.

Evidently the number of silver nuclei, formed during the impregnation for 15 minutes only, is sufficient (for the test-sections used in these experiments) *provided that the rate of the development is high enough.*

Obviously the silver nuclei as well as the combined silver ions are ubiquitous in an impregnated section though not evenly distributed.

Exp. 4. Treatments as in exp. 1 with the exception that the silver staining was developed in a solution containing 1 g. hydroquione, 0,5 g. borax and *0,5 g. anhydrous sodium sulphite* in 100 ml. of distilled water.

The staining was complete, satisfactorily differentiated, and useful. The nerve fibres stained intensely black.

Evidently DAVENPORT and KLINE (1938) correctly concluded that the sodium sulphite is a differentiating agent. SAMUEL (1953) stated that the combined silver ions (the reducible silver) could be removed from the section by treatment with a solution of sodium sulphite. He also concluded that there is a competition during the development between the silver-depositing action of the hydroquinone and the silver-removing action of the sulphite.

When a plain alkaline hydroquinone solution is used as in exp. 3., at least the bulk of the combined silver ions are reduced and deposited *in situ*. In the presence of sodium sulphite, on the other hand, the silver ions are made movable and able to increase the deposition centres at some sites and to decrease the deposition at other sites. Particularly at a slow rate of development the movable silver ions are also partly lost to the surrounding solution. Hence follows a differentiated staining.

Exp. 5. Treatments as follows. 1. Impregnation in 10 % aq. $AgNO_3$ for 24 hours at 37° C., 2. rinsing as above, 3. the pH-level elevated in the section by treatment for about 1 minute in 2 g. borax in 100 ml. of N/20 sodium hydroxide, 4. development in 1 g. hydroquinone and 5 g. anhydrous sodium sulphite in 100 ml. of the borax-sodium hydroxide solution for 5 minutes at 37° C., finally rinsing, toning, intensifying, and so on.

The staining was complete and similar to the staining in exp. 4. Most tissue elements stained too dark purplish, however. The cell nuclei and the nerve fibres were black but with a purplish tinge. This is the typical result of an 'overimpregnation'.

Evidently a great number of tightly placed silver nuclei form during the prolonged impregnation, and at the high rate of development the additional silver is forced out on too many deposition centres, forming too many and too small final silver (and gold) particles (compare Fig. 5 and 6).

Exp. 6. A series of test-sections mounted on 5 different slides were stained. Step 1. and 2. as in exp. 5., step 3: The pH adjusted in the sections by treatment for about 1 minute in a 2 % borax solution (without sodium hydroxide), step 4. The suitable rate of development was determined by treating one slide in each of the following 5 developers, namely

D_2 1 g. hydroquinone and 5 g. anhydrous Na_2SO_3 in 100 ml. of 2 % borax solution,

D_3 0,5 g. hydroquinone and 5 g. anhydrous Na_2SO_3 in 100 ml. of 2 % borax solution,

D_4 0,1 g. hydroquinone and 5 g. anhydrous Na_2SO_3 in 100 ml. of 2 % borax solution,

D_5 0,05 g. hydroquinone and 5 g. anhydrous Na_2SO_3 in 100 ml. of 2 % borax solution,

D_6 0,01 g. hydroquinone and 5 g. anhydrous Na_2SO_3 in 100 ml. of 2 % borax solution.

Finally rinsing, toning, intensifying, and so on.

The rate of the development is dependent on (1) the concentration (and the chemical composition) of the developing agent, (2) the pH, and (3) the temperature, to some extent also (4) on the chemical composition of the anion with

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which the silver ions are combined immediately previous to the reduction, the borate, and above all *the sulphite ions being particularly suitable.*

By decreasing the concentration of the hydroquinone a series of developers (D_1—D_6) with a gradually decreasing rate of development was most conveniently obtained. The highest rate, however, was obtained by addition of sodium hydroxide to the solution, i.e. by an increase of the pH as in exp. 5 (where the developer D_1 was used).

The pronounced purple tinge obtained in exp. 5 gradually faded when D_2, D_3 and D_4 were used instead of D_1. With D_5 the nerve fibres stained black while most non-nervous tissue elements stained light purplish. The striated muscle became dark grey after development with D_2—D_4. The sarcolemma stained dark purplish or even purplish black. In D_5 on the other hand, the sarcolemma stained light purplish only or remained unstained.

Evidently only some of the available silver nuclei serve as deposition centres during a development at a slow rate, and the size of some of these deposition centres is increased, forming a black instead of purplish staining.

Sometimes reticular fibres stain black after development at a slow rate. Therefore this mode of development, although highly recommendable for instance for the staining of the nerve fibres in the central nervous system of embryos and in many other cases, must be used with caution. In fact, with all silver staining methods including Schultze—Gros' and Bodian's method large final particles, i.e. a black staining, can be formed not only in the nerve fibres but also in other tissue elements (cell-nuclei, reticular fibres, endothelial, and basement membranes). Therefore in questionable cases other specific stainings should be used as a control (Palmgren, 1958). However, misleading co-stainings are avoidable by impregnation at a suitable pH-level and development at a high rate, or by pretreatment of the sections in acidified formalin (see below).

Exp. 7. Treatments as in exp. 5 with the exception that the sections were impregnated in 10 g. $AgNO_3$ in 100 ml. of N/1000 nitric acid, thus with an increased concentration of hydrogen ions.

During the impregnation the sections in exp. 5 and 6 stained light yellowish brown. In exp. 7 on the other hand the sections remained almost quite unstained. After development at the highest rate (with D_1) the staining was complete, differentiated, and useful but rather poor. Therefore another section of the same series was treated in the same manner except that the procedure was carried out up to and including the (first) development and then repeated from the impregnation (for 15 minutes only) to a (second) development. The final result was similar to the staining after the development in D_4 in exp. 7, in fact more satisfactory, as the sarcolemma remained quite unstained.

Generally a development at a high rate is preferable, because the loss of silver ions to the surrounding fluid is minimized. A high rate of development, however, requires a comparatively small number of silver nuclei, otherwise the too nume-

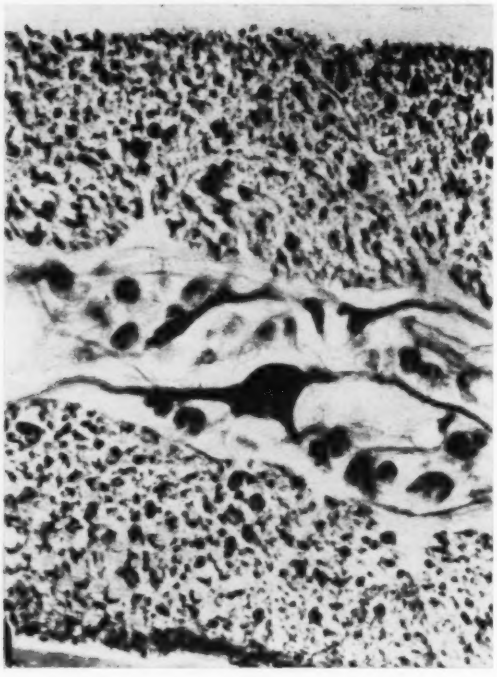

Fig. 9. Part of the spinal cord of hag-fish from the same series of sections as in Fig. 8. Stained by the formula I_3 5 minutes D_1 (see the text). 500 ×.

rous deposition centres produce small final particles and a more or less purplish staining throughout.

In Bodian's and Holmes's methods the formation of too many silver nuclei (deposition centres) is prevented by the extremely low concentration of silver ions during the impregnation. In exp. 7 competitory hydrogen ions serve the same purpose.

However, to prevent the formation of too many deposition centres the impregnation *time* could also be shortened. When I_2 is used the time could and should be shortened to 15 minutes, and an impregnation in I_3 for 5 minutes only delivers after a development at the highest rate (D_1) excellent staining results (for Cyclostomes), (Fig. 9).

Exp. 8. Treatments as follows. 1. Impregnation in 10 % aq. $AgNO_3$ for 15 minutes at 37° C., 2. rinsing through 5 changes of distilled water, 3. the pH within the sections elevated by treatment for about 1 minute in 2 % borax solution, 4. development in the developer D_3 *for 3 seconds* at 37° C, then the development at once interrupted by treatment in N/100 nitric acid, 5. rinsing, toning, intensifying, and so on.

Some of the nerve fibres in the spinal cord and in the peripheral nerve stems stained black, others remained poorly stained or unstained, (Fig. 10). The mucous cells, the red blood corpuscles, and parts of the striated muscle stained pur-

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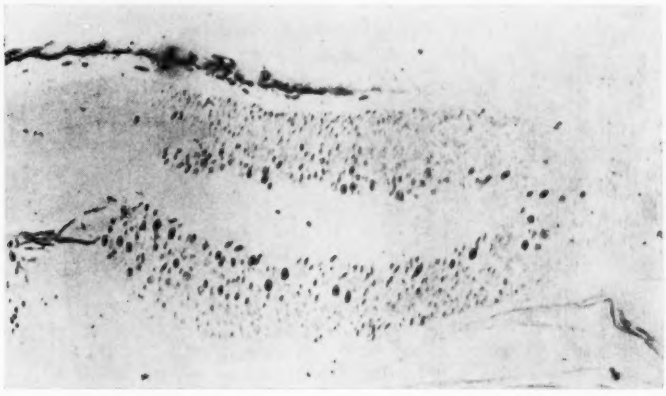

Fig. 10. Part of the spinal cord of hag-fish from the same series of sections as in Fig. 8. The result of experiment 8 (see the text). The development interrupted after 3 seconds, showing that the deposition of silver does not occur simultaneously in all tissue elements. 150 ×.

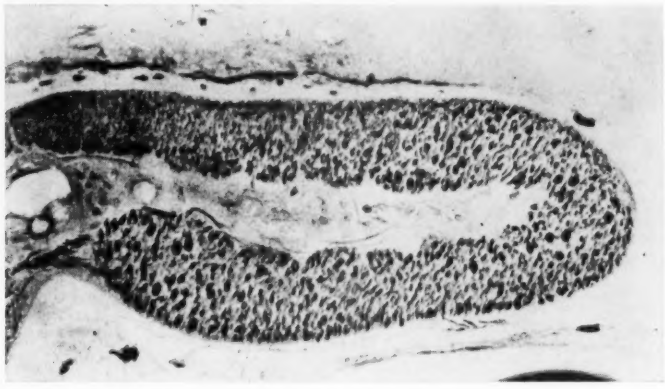

Fig. 11. Part of the spinal cord of hag-fish from the same series of sections as in Fig. 8. Stained in the same way as the section in Fig. 10 but with undisturbed development for 5 minutes (see experiment 8). 150 ×.

plish, otherwise the sections were quite unstained. By an undisturbed development with D_3 (for 5 minutes at 37° C), on the other hand, other sections of the same series, otherwise treated in the same way, stained completely, (Fig. 11).

Evidently all tissue elements do not stain simultaneously. Some elements stain at an earlier stage of the development than others.

Probably the deposition of additional silver starts where the silver ions are most concentrated or most available for the developer. Once formed the *first* silver depositions autocatalyze the further depositing, which means a consumption

Fig. 12. Frozen section at 80 microns from a human molar, fixed in Bouin's fluid. Nerve fibres (and blood vessels) in the pulp cavity stained by the formula 1_2 5 minutes D_1 with rinsing in distilled water for 1 hour after the impregnation (see the text). 150 ×.

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of silver ions in their close vicinity and thus a decreased deposition or none at all on the surrounding silver nuclei.

As the very first deposition of silver and the rate of development are most important, it is always necessary to adjust the pH-level within the sections previous to the development, and then, without rinsing, to transfer the sections to the developer. In this way (thus by pre-treatment in the solvent of the developer) the pH-level is controlled and kept constant from the very start of the development. In fact this pre-treatment (for less than 1 minute) effectively replaces the second impregnation (for 24 hours) with the alkaline silver nitrate solution used in HOLMES's method (1947).

After the completed development the sections should be rinsed in 50 % alcohol instead of in distilled water, because otherwise the sections sometimes get loose from the slide.

Summary

By treatment (impregnation) of a section in a silver nitrate solution silver ions combine with tissue substances and are partly reduced to silver nuclei (LIESEGANG, 1911). The concentration of the silver ions, the pH, the time, and the tempera-

ture influence this process (DAVENPORT, MCARTHUR and BRUESCH, 1939). Each of these factors can be varied within wide limits, because they are inversely proportional to each other, and one factor can be corrected by adjustment of another. Their relative values, on the other hand, determine the result within narrow limits. The same is true of other factors influencing the complete staining procedure. The amount of combined silver ions and silver nuclei increases with the impregnation time until a maximum is reached, which is dependent on the concentration of the free silver ions and the pH-level in the solution (cf. ROMANES, 1950, LIPP, 1951, and PETERS, 1955 a). The distribution can be roughly demonstrated by the methods described above. Impregnated nerve fibres and cell nuclei contain combined silver ions but only comparatively few silver nuclei. The reverse is true of the cytoplasm and other tissue elements (Fig. 1 and 2). In the present method (as well as in Bodian's and Holmes's) the final silver particles are replaced by gold particles, and the gold staining is intensified. Generally the gold staining is either black or purplish. The black (specific) staining consists of comparatively large, tightly placed globules (Fig. 4 and 5) and the purplish of small scattered granules (Fig. 4 and 7). When the combined silver ions are deposited *in situ* by a plain alkaline hydroquinone solution (see above, exp. 3) some tissue elements stain black other purplish. Thus, in the main, the size of the particles is determined already by the chemical and physical state of the tissues (and their contents of formalin), or, in other words, by the ratio between the concentration of the combined silver ions and the amount of silver nuclei at different sites. An always limited amount of combined silver ions deposited on a great number of silver nuclei or deposition centres form small particles and a purplish stain, whereas large particles and a black staining form at sites where a comparatively large amount of combined silver ions is deposited on a small number of deposition centres. In the present method the combined silver ions are made movable with sodium sulphite during the development (SAMUEL, 1953). Probably the deposition of additional silver starts where the silver ions are most concentrated or most available for the developer. Once formed the first deposition autocatalyzes the further depositing of silver, which means growing deposition centres and consumption of the movable silver ions in their close vicinity, and thus a more differentiated, more intensely black and more light purplish staining. By a slow rate of development the differentiation get time to be more accentuated. In the present method the amount of combined silver ions is adjusted by small but important variations in the pH-level, and the amount of silver nuclei also by using a suitable, short impregnation time. The rate of development is adjusted mainly by variations in the concentration of the hydroquinone. Thus, the present method is adaptable to the different stainability of different specimens. Probably no rigid method will ever have a sufficiently wide range of utility.

The Toning and the Intensifying

When the developed and rinsed silver staining is treated (toned) with a solution of gold chloride, the yellowish brown tinge of the silver staining disappears almost completely within about 15—30 seconds. The metallic silver particles, which constitute the silver staining, are replaced by gold particles, and simultaneously silver chloride forms, thus $3\ Ag + AuCl_3 = Au + 3\ AgCl$ or if acidified, so-called yellow gold chloride is used, $3\ Ag + HAuCl_4 = Au + 3\ AgCl + HCl$. Thus, each silver particle is transformed into an Au—AgCl conglomerate, since silver chloride is only very slightly soluble in water. These composite particles act as deposition centres when the final gold staining is developed by the reduction of the remaining auric ions within the sections. The AgCl-component of the deposition centres is not indifferent to this development or 'intensifying'. If the silver chloride is removed by fixing *previous to* the intensifying, the otherwise black gold staining of the nerve fibres becomes purplish. This purplish staining could not be made black by repeated toning and intensifying. Thus, naturally, the size of the Au-component is smaller than the size of the composite Au-AgCl particle.

Contrary to this, when the silver chloride is removed by fixing *after* a completed toning and intensifying (and rinsing), a black or purplish-black gold staining could be made much intenser *by repeated toning and intensifying*. This '*additional intensifying*' (which can be used whenever desired, thus even for mounted and covered sections after removing the cover-glass and the balm and the usual passage through the alcohol series to water) may be useful in some cases, for instance for staining of sections from the central nervous system. However, the additional intensifying means an enlargement of all the gold particles in the section, and therefore the differentiation is not improved. The author used the additional intensifying in rare cases only, for instance for the determination of the distribution of the silver nuclei (see above).

About the same result could be obtained by the reduction (instead of the removing) of the silver chloride with hydroquinone, followed by a repeated toning and intensifying (Palmgren, 1958), but the additional intensifying described above is simpler and more convenient.

Davenport and Kline (1938) introduced a 'second reduction' after the toning by an amidol-developer, which reduced the silver chloride and the auric ions simultaneously. In the present author's experience it is preferable to reduce the auric ions with acids.

For this purpose various acids, including oxalic acid, were already used by many authors during the last decades of the 19th century. Walter (1913) employed acetic acid in glycerol, and Bodian (1936) an aqueous oxalic acid solution at room-temperature.

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The gold staining is much improved, however, by intensifying in an *alcoholic* solution of oxalic acid *at 37° C.*

The Procedure

A. THE FIXATION

Fixatives containing osmic acid or dichromates should not be used for material intended for silver staining of nerve fibres. Osmic acid irreversibly blocks the silver stainability, dichromates do not but must be *completely* removed from the tissues, preferably with the potassium permanganate-oxalic acid-potassium sulphite solutions introduced by PAL (1886). However, during this treatment mounted paraffin sections get loose from the slide.

The Bielschowsky technique requires a fixation or at least a pre-treatment in formalin, whereas the Cajal-methods generally produce the best results after fixation in alcohol or solutions which do not contain formalin.

The reactions between formaldehyde and the proteins are complicated and far from completely understood. However, they include i.a. methylene cross-links ($-CH_2-$) between different amino—and imidazole groups (FRAENKEL—CONRAT and MECHAN, 1949). Methylol ($-CH_2OH$) or dimethylol groups also form (NEURATH and BAILEY, 1953).

Some of these reactions are reversible, which means that formaldehyde is steadily liberated from the tissues (LIPP, 1951) and increases the redox-potentials. Besides, the cross-linkage influences the physical state of the tissues. Thus, in several respects the formalin interferes with the silver stainability.

The nerve fibres in material (from vertebrates) fixed *without formalin* in alcoholic fixatives or in sublimate-acetic acid (PALMGREN, 1955) stain very satisfactorily black with the present method. For the central nervous system this type of fixatives (as for instance Carnoy's fluid) is particularly suitable. When used for the peripheral nervous system the cell nuclei, sometimes also the fibrocytes and cell granules are too heavily stained. This undesired co-staining could be effectively suppressed, however, by pre-treatment of the sections with acidified formalin (see below).

Among fixatives which *contain formalin* Bouin's fluid is advantageous because it produces a satisfactory fixation from a histological point of view. A suitable fixative for the purpose is also Bodian's fluid, formula 2 (BODIAN, 1937), which is a compromise between the two main types of fixatives (fixatives with formalin and alcoholic fixatives without formalin) as it contains alcohol but also a small amount of acidified formalin. Also after fixation in Bodian's fluid a pre-treatment of the sections with acidified formalin is advisable provided that reticular fibres or basement membranes are co-stained.

Plain formalin is less suitable. If it is used, it should be used as a 10 % solution of formaldehyde, thus commercial formalin diluted with water in the proportion 1 : 3.

As is well-known, the soluble and oxidizable lipids interfere with the silver staining. Undoubtedly such lipids are most effectively removed by fixation in alcoholic fixatives. In other fixatives the lipids are probably slowly destroyed. Thus, it is advisable always to fix *for a sufficiently long time,* generally at least 48 hours (or for weeks or months when plain formalin is used), and not to perform the paraffin embedding procedure too rapidly. Generally the specimens should be treated through the alcohol series for at least 24 hours in each concentration. Specimens should not be stored in alcohol for very long periods (years), but should be embedded and stored in paraffin blocks.*

For material fixed in Carnoy's or similar fluids it is recommendable to use methyl-benzoate (or similar compounds) after the treatment in absolute alcohol and previous to the treatment in benzene (toulene or xylene), because otherwise such material might become brittle and cause unnecessary difficulties at the sectioning.

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Since the fixative greatly influences the silver stainability and it is impossible to foresee with certainty what fixative would be preferable for each particular specimen, fresh material intended for silver staining of nerve fibres should be fixed, if possible, in three different fixatives, namely Carnoy's fluid, Bouin's fluid, and Bodian's fluid, formula 2 (BODIAN, 1937), or in similar fixatives. Generally Carnoy's fluid is preferable for parts of the central nervous system of vertebrates, and also very useful for peripheral nerve fibres, while Bouin's fluid and Bodian's are more useful for the peripheral than for the central nervous system.

B. THE SECTIONS

Generally paraffin sections at 15 microns, mounted on slides, are the most suitable. For particular purposes, for instance when it is desirable to demonstrate the connections and ramifications of nerve fibres, also thicker (up to 50 microns) mounted paraffin sections could be used.

The mode of mounting paraffin sections on glass-slides is not quite unimportant. The slides should be coated with a thin layer of Mayer's egg-albumen (fresh egg white dissolved in about the equal volume of pure glycerol, with some pieces of solid camphor to prevent putrefaction). Most of the egg-albumen should be washed away from the slide with distilled water. Some more distilled water should be added and the sections transferred to the water surface, warmed until a sufficient stretching is obtained, and finally, after removal of the excess water, dried for 24 hours at 37° C.

* A particularly suitable sort of paraffin wax (melting point 56—58° C) is manufactured by the firm E. Merck in Darmstadt. This paraffin has the number 7153.

Complete avoidance of every impurity is essential in this case as well as in the entire procedure.

Still thicker sections (100—200 microns), obtained with the freezing microtome, could be mounted on slides between thin layers of collodion or, preferably, be handled separately and transferred to the different fluids with glass-spatulae. Even whole, not sectioned membranes and similar material could be stained, provided that tightly placed nerve fibres or other structures do not make the stained preparation untransparent.

Mounted, dried paraffin sections should not be stored for long periods because sunlight and vapours in the air (for instance vapour of formalin, acids, or ammonia) sometimes very markedly influence the stainability. Sunlight and remaining lipids reduce the picric acid in sections from material fixed in Bouin's fluid, and the reduced compounds react with the proteins. The lemon-yellow tinge of the sections changes to an orange-yellow, and such derivatives cannot be removed from the sections. In spite of this, the nerve fibres are stainable but generally not satisfactorily. Frozen sections or whole, thin preparations, which are stored in alcohol could lose their stainability after about a month (PALMGREN, 1951).

The very best results are always obtained by staining of *fresh-made* paraffin sections.

C. PRE-TREATMENTS

1. *For sections from material fixed in fixatives which contain formalin.*

The yellow tinge of sections from material fixed in Bouin's fluid, i.e. picric acid, should *always* be removed in ammoniated alcohol (about 1 ml. of ammonia in 100 ml. 70 % alcohol) and the sections afterwards rinsed through several changes of distilled water previous to the impregnation.

Although a certain small number of silver nuclei or deposition centres is a necessity, and a great number if placed in the non-nervous tissues does no harm as it only causes a more or less intense purplish counter-staining, a great number placed within the nerve fibres sometimes makes it impossible to obtain a specific (black) staining, because the available silver ions are unable to form large particles when dispersed on to a great many deposition centres. The formation of too many silver nuclei, an 'overimpregnation', can be suppressed for instance by shortening the impregnation time or by increasing the hydrogen ion concentration, but in spite of such precautions some material, for instance embryos in early stages are easily overimpregnated. In such cases a very favourable effect is obtainable simply by pre-treatment of the sections in distilled water (with a piece of camphor) for 24 hours at 37° C. Some of the reactions between formaldehyde and proteins are reversible, and when formaldehyde and maybe also other reducing substances are removed, the redox-potentials and hence also the formation of silver nuclei are diminished.

However, it should be noticed that a decreased amount of formalin in combination with a slow rate of development sometimes increases the stainability of the reticular fibres. This stainability can be suppressed by pre-treatment with acidified formalin.

Thus, for different purposes the concentration of formaldehyde in the sections can be adjusted by pre-treatment either in distilled water or in the acidified formalin described in the following.

2. *For sections from material fixed in fixatives which do not contain formalin.*

The cell nuclei and some other structures often stain too heavily. Such disturbing stainings should be suppressed by pre-treatment of the sections in a solution of 5 ml. formalin, 5 ml. glacial acetic acid and 90 ml. distilled water for 1—24 hours at 20° C. The pre-treated sections behave in about the same way as sections from material fixed in fixatives with formalin. The nerve fibres, however, stain much more satisfactorily. Undoubtedly the formaldehyde reacts also with fixed proteins.

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3. *For all sections.*

If accidentally sections show tendencies to get loose from the slide, it should be transferred to absolute alcohol and coated with a *thin* layer of collodion (½ % celloidine in absolute alcohol and ether, equal volumes).

D. THE STAINING PROCEDURE

a. *Stock Solutions.*

1. N/10 nitric acid and N/10 sodium hydroxide, both made by pouring the contents of commercial ampoules with standardized volumetric solutions into glass-stoppered volumetric flasks.

2. N/1000 nitric acid made with great accuracy by dilution of N/10 nitric acid using burette and volumetric flask.

3. N/20 sodium hydroxide made by dilution of N/10 sodium hydroxide with distilled water.

4. Aqueous solution of 0,02 % pyridine, made by pouring 0,2 ml. of pyridine into a volumetric flask and diluting with distilled water to 1 litre volume.

5. 20 g. borax ($Na_2B_4O_7.10\ H_2O$) in 1000 ml. of N/20 sodium hydroxide.

6. 20 g. borax in 1000 ml. of distilled water.

7. 1 g. yellow gold chloride ($HAuCl_4$) and 6 drops of glacial acetic acid in 200 ml. of distilled water.

8. 5 g. oxalic acid in 1000 ml. of 50 % alcohol.

9. 5 % aqueous sodium thiosulphate.

All the chemicals should be C.P. or 'pro analysi' products.

b. *The Main Sequence of Treatments.*

1. Deparaffinize in toluene (or xylene) and transfer through the alcohol series (i.e. (1) absolute alcohol + toluene equal volumes, (2) pure absolute alcohol, and (3) 95 % alcohol), and through 3 changes of distilled water. Sections from material fixed in Bouin's fluid should be transferred from the 95 % alcohol to ammoniated alcohol (1 ml. ammonia in 100 ml. of 70 % alcohol), and after destaining rinsed in tap water and through 3 changes of distilled water.

2. Pre-treat, provided that adaptation trials show that pre-treatments (see above) are desirable or necessary, either in distilled water (with a piece of camphor) for 24 hours at 37° C., or in a solution containing 5 ml. formalin, 5 ml. glacial acetic acid and 90 ml. distilled water for 1—24 hours at 20° C. Then rinse through 3 changes of distilled water.

3. *Impregnate in* one of the following three silver nitrate solutions, which can be kept in the incubator and used daily for a fortnight, namely I_1 10 g. silver nitrate in 100 ml. of N/1000 nitric acid,
I_2 10 g. silver nitrate in 100 ml. of distilled water,
I_3 10 g. silver nitrate in 100 ml. of 0,02 % aqueous pyridine (stock solution 4) *for a suitable time,* namely for I_1 generally overnight, for I_2 generally 15 minutes and for I_3 2—10 minutes for material fixed in formalin-containing fixatives or pre-treated in formalin or otherwise overnight,
at 37° C. For further details see below.

4. Rinse through 5 changes of distilled water for about 2 minutes in all at about 20° C. Very thick sections should be rinsed for about 1 hour.

5. Adjust the pH-level within the sections by treatment in a portion of the solvent of the developer (stock solution 5 for D_1, stock solution 6 for D_2—D_6) for ½—1 minute at about 20° C.

6. Without previous rinsing develop in one (generally the first) of the following developers, which can be kept in the incubator and used daily for a week, namely
D_1 1 g. hydroquinone and 5 g. anhydrous sodium sulphite in 100 ml. of the stock solution 5,
D_2 1 g. hydroquinone, D_3 0,5 g. hydroquinone, D_4 0,1 g. hydroquinone, D_5 0,05 g. hydroquinone, D_6 0,01 g. hydroquinone and 5 g. anhydrous sodium sulphite in 100 ml. of the stock solution 6, (see p. 250).
for 5—10 minutes at 37° C. Agitate the slide during the first few seconds of the development.

7. Rinse through 3 changes of 50 % alcohol for 5 minutes or for a longer time at about 20° C. Sections coated with collodion should be rinsed for at least 15 minutes.

7 a. If a repetition is desirable, rinse in distilled water for 15 minutes, impregnate (step 3) for 10 minutes and repeat the whole procedure.

8. Tone with a few drops of the gold chloride solution (stock solution 7) for 15—30 seconds or until the yellowish brown colour of the sections disappears.

9. Rinse briefly in water and intensify in the oxalic acid solution (stock solution 8) for 2—2 ½ minutes at 37° C. The solution can be used repeatedly.

10. Rinse under the tap, fix in 5 % aqueous sodium thiosulphate for about 10 seconds, rinse again, dehydrate and cover.

10 a. The additional intensifying (see above) means a repetition from step 8 after the fixation and rinsing.

E. THE ADAPTATION OF THE METHOD TO DIFFERENT PURPOSES

Since the stainability of the nerve fibres in different species and, sometimes, also of different nerve fibres in the same species varies considerably, a rigid method will probably never have a sufficiently wide range of utility. Not even the range of Bodian's method is wide enough. Therefore, the present method is not rigid but adaptable and should consequently be adapted. For this purpose a series of test-sections (containing nervous tissue) should be used. One or two sections on each slide, and generally about 15 slides are sufficient.

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It is provided that sections from material fixed in Bouin's fluid are destained with ammoniated alcohol, and that sections fixed in Carnoy's fluid are pre-treated with the acid formalin solution (see above).

The variables are (1) the impregnation type I_1—I_3, (2) the impregnation time, and (3) the rate of development (D_1—D_6).

The adaptation should be started with five slides, one for each of the following formulae, namely I_1 15 minutes D_1, I_2 15 minutes D_1, I_3 10 minutes D_1, I_1 24 hours D_1, I_2 24 hours D_1. If the final gold staining is too purplish the impregnation time should be shortened, down to 2 minutes if necessary.

In most cases one of these formulae appears to be superior to the others. The result of the preferable formula can be improved

1) if the staining still becomes too purplish or the differentiation is unsatisfactory by determination of the suitable rate of development using successively the developer D_2, D_3, and so on, instead of D_1,

2) if the final staining is poor but otherwise satisfactory by repetition of the procedure at step 7 a (using a new slide),

3) if a satisfactory staining of some, for instance the motor nerve fibres is obtainable with one formula and another formula is preferable for other, for instance autonomic nerve fibres in the same section these formulae can be combined by using the one formula to and including step 7 a and the other for the repetition from step 3,

4) if Bouin's fluid has been used as a fixative and the staining is too purplish by pre-treatment in distilled water for 24 hours at 37° C.,

5) if reticular fibres are co-stained by pre-treatment in the acidified formalin (see above).

If sections from material fixed in Carnoy's fluid are stained *without* pre-treatment the impregnation time should be 24 hours, at least at the first trials.

Failure at this stage of the adaptation to obtain a satisfactory result, that is to say a distinct black staining of the nerve fibres and a light purplish counter-staining, is dependent either on a mistake made at the selection of the formula, which at first sight appeared to be superior to the others, or on the fact that an unsuitable fixative or fixation technique has been used.

In the former case another impregnation type should be tried and the result improved in the same way as described above.

In the latter case another fixative should be tried and used as recommended above.

The result of the adaptation of the method for some specimens is given below.

(Abbreviations: B = Bouin's fluid, B_2 = Bodian's fluid formula 2, C = Carnoy's fluid, H_2O = distilled water with a piece of camphor, a.f. = acidified formalin, Rep. = repetition of the procedure.)

Specimen	*Fixation*	*Pre-treatment*	*Formula*
Lancelet, transv.sections	B	H_2O	I_1 24 hours D_1
	B_2	—	I_1 24 hours D_2
Hag-fish, transv.sections	B	H_2O	I_1 24 hours D_1 Rep.
	B_2	—	I_3 5 min. D_2 or I_2 15 min. D_1
Lamprey, transv.sections	B	—	I_3 10 min. D_1 or D_2
Eel, heart, eye, intestine	B	H_2O	I_1 24 hours D_1
Eel, brain	C	a.f.	I_3 10 min. D_1
Eel, intestine	C	a.f.	I_1 15 min. D_1
Roach, embryos 6—15 mm.	B	H_2O	I_1 5 min. D_1 or I_1 24 hours D_3
Frog, adult, head	C	—	I_2 24 hours D_1
Frog, young, transv.sect.	B	H_2O	I_1 24 hours D_1
	B	—	I_1 24 hours D_3
Newt, transv.sections of head	B	H_2O	I_1 24 hours D_1
Tortoise, brain	C	a.f.	I_1 24 hours D_1 Rep.
Tortoise, blood vessels	B_2	a.f.	I_2 24 hours D_4
Tortoise, intestine	B	H_2O	I_1 24 hours D_1
Fowl, eye	B	H_2O	I_1 24 hours D_1 Rep.
Fowl, heart	B_2	—	I_1 24 hours D_1 or I_3 10 min. D_1

Specimen	*Fixation*	*Pre-treatment*	*Formula*
Fowl, intestine	C	a.f.	I_1 24 hours D_1
Cat, tongue	C	a.f.	I_2 10 min. D_4
Dog, jaw and teeth	B	—	I_2 10 min. D_1 or I_2 15 min. D_3
Cow, blood vessels	B	H_2O	I_2 24 hours D_2 or D_3
Rat, blood vessels	B	H_2O	I_3 5 min. D_1 or I_2 hours D_1
Rat, blood vessels	B_2	—	I_3 2 min. D_1 or I_2 15 min. D_3
Monkey, blood vessels	B, B_2 C	—	I_3 10 min. D_1 or D_3
Sheep, embryo, forebrain	C	—	I_2 24 hours D_6
Man, embryo, head	B	—	I_3 3 min. D_1
Man, tooth, sections at 80 microns	B	—	I_2 5 min. (H_2O 1 hour) D_1, Fig. 12
Man, joint capsule	B	—	I_2 15 min. D_1

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The Cytology of the Caecal Epithelial Cells of *Perca*

by

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Introduction

The morphology of the so-called "pyloric caeca", *appendices pyloricae,* of various teleosts has been described by, among others, JACOBSHAGEN (1915), PERNKOPF and LEHNER (1937), RAUTHER (1940), SUYEHIRO (1941), RAHIMULLAH (1945), AL-HUSSAINI and KHOLY (1954), and NAGAR and KHAN (1958). The authors have shown that the caecal mucosa is composed of columnar epithelium cells with interspersed goblet cells. Occasionally some wandering blood cells are found in the epithelium.

Because the caecal mucosa is very like that of the intestine it is generally believed that the function of the appendages is mainly or exclusively resorptive. SCHNAKENBECK (1955, p. 737) writes: "Man denkt die Pylorusanhänge als eine Vergrösserung der Darmoberfläche" and BARRINGTON (1957, p. 122) says: "It has been suggested that they are merely an adaption which increases the surface area of the intestinal epithelium of fish in which the body cavity limits the length of the intestine itself."

However, several authors, among them MILNE EDWARDS (1860), MOREAU (1881), MACALLUM (1886), and BEN DAWES (1929), have suggested a glandular function of the caecal epithelial cells. KRUKENBERG (1882) suggested that the function of the caeca in *Perca* is to produce goblet cell mucus to the chyme. JACOBSHAGEN (1915) has even described intestinal (Lieberkühn) glands in some

species *(Gadidae)*. Various enzymes have been found in the caeca (MILNE EDWARDS 1860, MOREAU 1881, KRUKENBERG 1882, BLANCHARD 1882, TANI 1940, WEINREB and BILSTAD 1955) and of these, lipase, proteases (trypsin) and diastase are in different species said to be produced by the caecal mucosa (STIRLING, RAHIMULLAH, AL-HUSSAINI and KHOLY, GEORGE and DESAI (1947)).

Another rather doubtful point in this connection is the existence of ciliated cells in the caeca. EDINGER (1876), KRUKENBERG (1878), MACALLUM (1886), and others (see RAHIMULLAH 1945), have in some cases described ciliated cells as ordinary members of the caecal mucosa or concentrated near the entrance of the caeca.

Because of this often rather confusing information regarding the cellular elements of the caeca it seemed to be of interest to undertake a detailed analysis in a single species of the fine structure of the cells as revealed by the electron microscope and of possible secretory cell inclusions with the aid of some histochemical reactions.

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Material

The common perch, *Perca fluviatilis,* was chosen as material for this investigation. For some preparations fishes were killed which had previously been well fed and could be assumed to be in a condition of digestion. Every slide was prepared to contain one row of sections from each of the distal, median and proximal parts of a caecum as well as one row of sections through the intestine a short distance caudad of the attachment of the caeca. This arrangement made a direct comparison possible between intestinal and caecal mucosa with all the techniques used. A series of longitudinal sections through a caecum was used for the histological studies.

Methods

Methods without references in the literature list in this paper have been taken from the usual text-books of ROMEIS (1948), CASSELMAN (1959) and PEARSE (1960). All the methods mentioned here were applied on the different caecum sections as well as the gut controls. In cases when no further references to a method are made in the text, this means that no conclusive results could be obtained with the technique in question.

ROUTINE METHODS

Fixation according to Bouin, Zenker, Helly, Flemming ("strong"), Carnoy (6 : 3 : 1) and Champy as well as in formol-picric acid-trichloroacetic acid

(HALMI 1952) and 4—10 % neutral formol with 1 % calcium chloride. Paraffin sections, 5—8 μ, were stained in azan and iron haematoxylin according to Heidenhain, chrome haematoxylin, paraldehyde fuchsin and "trichrome" according to Gomori and the method for connective tissue fibres according to LUBOW and DOLNICK (1951).

SPECIAL METHODS

Impregnations of reticular fibres according to Gömöri and of Golgi apparatus after Aoyama (see BAKER 1951 a). Vital staining of mitochondria with janus green.

HISTOCHEMICAL REACTIONS

Periodic acid/Schiff reaction with and without previous diastase digestion, Best's carmine method, mucicarmine, mucihaematein and alcian blue for carbohydrates. Millon reaction, ninhydrin/Schiff, Astra blau with and without previous permanganate oxidation (OLSSON 1958) and pepsin and trypsin digestions for proteins. Nile blue and Sudan black B for lipides, the latter on paraffin sections as well as after a carbowax technique (FIRMINGER 1950). Nucleal test (Feulgen) and ribonucleic acid test with ribonuclease controls (after ABOLINS 1952). Azur I for metachromasia. Gomori's Ca/Co method for alkaline phosphatases and Gomori's lead nitrate method for acid phosphatases, the former on fresh and the latter on formalin fixed freezing sections.

ELECTRON MICROSCOPY

Small intestinal and caecal mucosa pieces were fixed in 1—2 % OsO_4 buffered at pH 7.4 and with the osmolarity of the fixing fluid kept at 0.40—0.45. Sectioning of the methyl-/butylmetacrylate (1 : 4) imbedded material on a LKB Ultrotome. Observations and micrographs with the aid of an Akashi TRS-50 electron microscope (50kV) with 100 μ anode and 50—100 μ objective aperture diaphragms.

Histology of the caeca

Most authors seem to agree that no conspicuous differences exist in the histology of the appendages and that of the intestine. *Perca,* which was studied by KRUKENBERG (1878) and JACOBSHAGEN (1915), is apparently no exception from this rule, but a few observations seem to be worth mentioning in this connection.

The caecal mucosa is richly folded especially in the proximal part of the caecum, were the folds often give the impression of branching and anastomo-

sing in the sections. The mucosal muscle layer is as well developed as that of the intestine. The submucosa contains a dense network of reticular fibres, which also penetrate the stroma of the folds. The stroma is also richly vascularized. The muscularis layers have the same arrangement as those of the intestine but the circular muscle layer seems to be less developed in the caeca, while the longitudinal layer has about the same thickness in the two regions.

Cytology of the caecal cells

GOBLET CELLS

The number of caecal goblet cells decreases in the distal portion of an appendix. Only in the proximal portion of it do they seem to be as abundant as in the intestine. In the caeca as well as in the intestine the goblet cells are more numerous on the tips of the folds than in the pockets between these structures. The goblet cells have the usual form and an ultrastructure which closely corresponds to the description given by TAYLOR (1959). They are apparently provided with microvilli in the inactive phase, which eventually disappear when the cells become mature and contain mucinogen globules (Fig. 5). The mucinogen is finally released at the cell apex into the caecal lumen.

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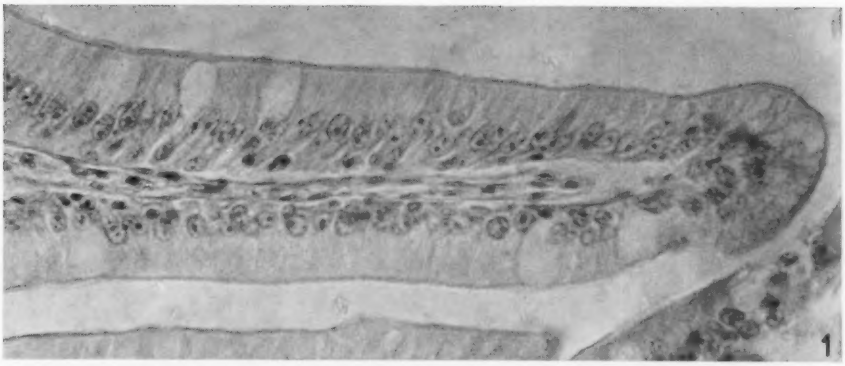
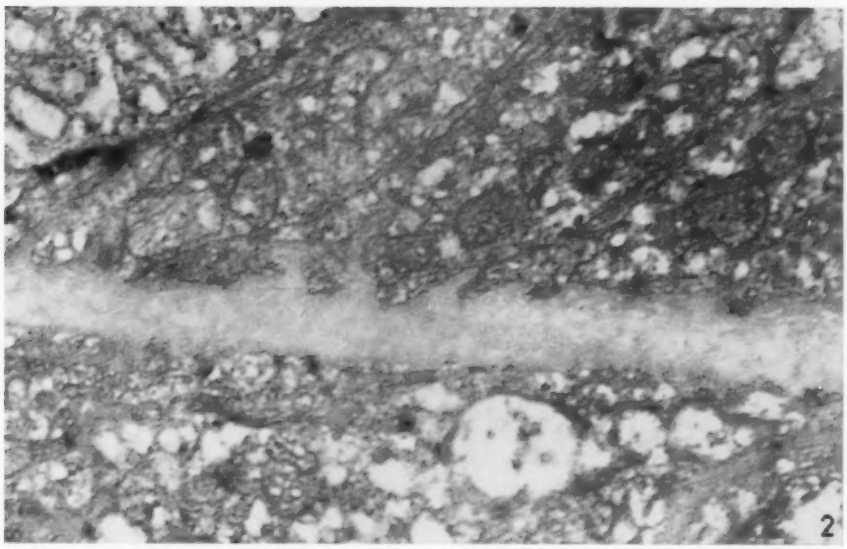
COLUMNAR CELLS

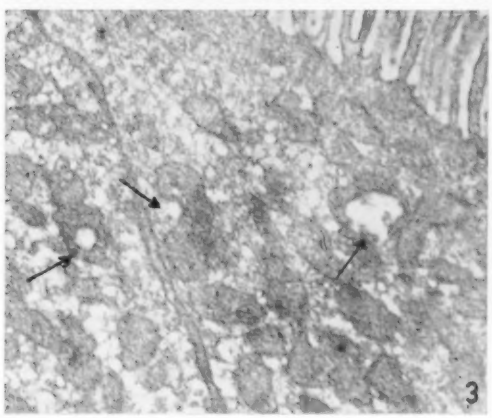
The light microscope observations on the caecal columnar cells suggest the occurrence of a striated border, which stains but faintly with most of the methods used here (Fig. 1). It has not been possible to trace cilia or basal corpuscles anywhere in the caeca. The slightly elongated nuclei contain distinct nucleoli and are oriented along the longitudinal axis of the cells. The cytoplasm portion below the striated border and apical to the nucleus usually stains strikingly darker than the rest of the cytoplasm.

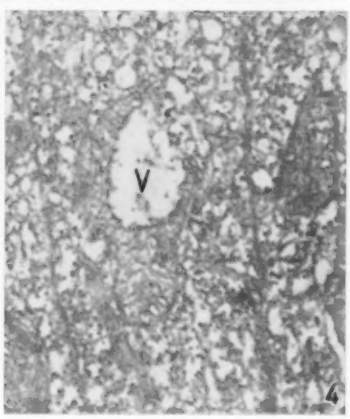
Free cell border. The electron micrographs show that the columnar cells are provided with a striated border which consists of slender microvilli measuring about 1.3 $\mu \times 80 m\mu$ in size. Sometimes several microvilli arise from a single protoplasm projection and thus give the impression of being proximally branched (Fig. 5). In some cases dense plasma streaks extend from the microvilli for a distance into the apical cell cytoplasm (see FAWCETT 1958). It has never been possible to see a caecal cell which carries cilia.

Other cell surfaces. The cell membranes are apically provided with not very conspicuous terminal bars. Basally they terminate on a thickened epithelial basement membrane without folding. The basement membrane appears structureless in the micrographs but it is often provided with small projections towards the epithelial cells (Fig. 2).





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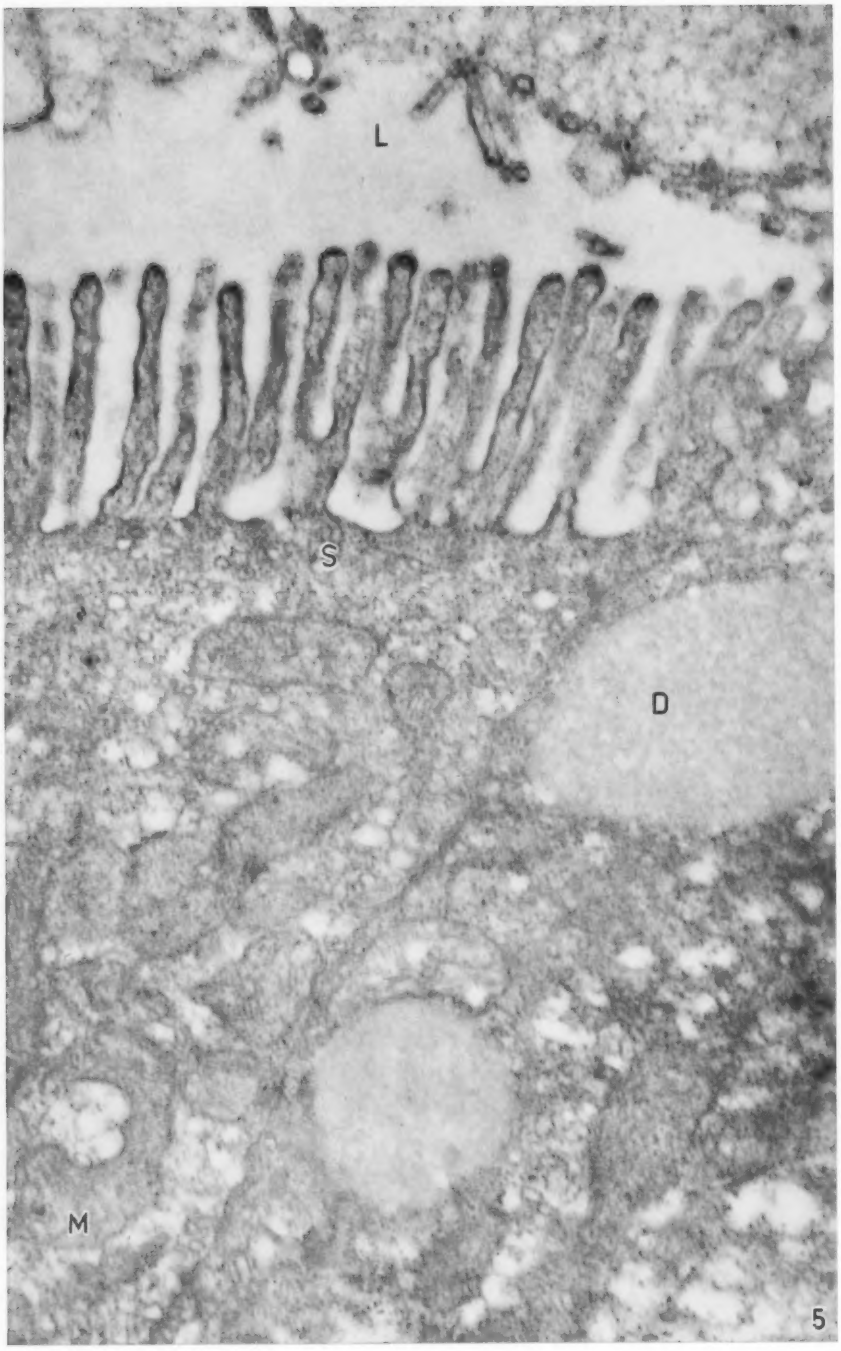

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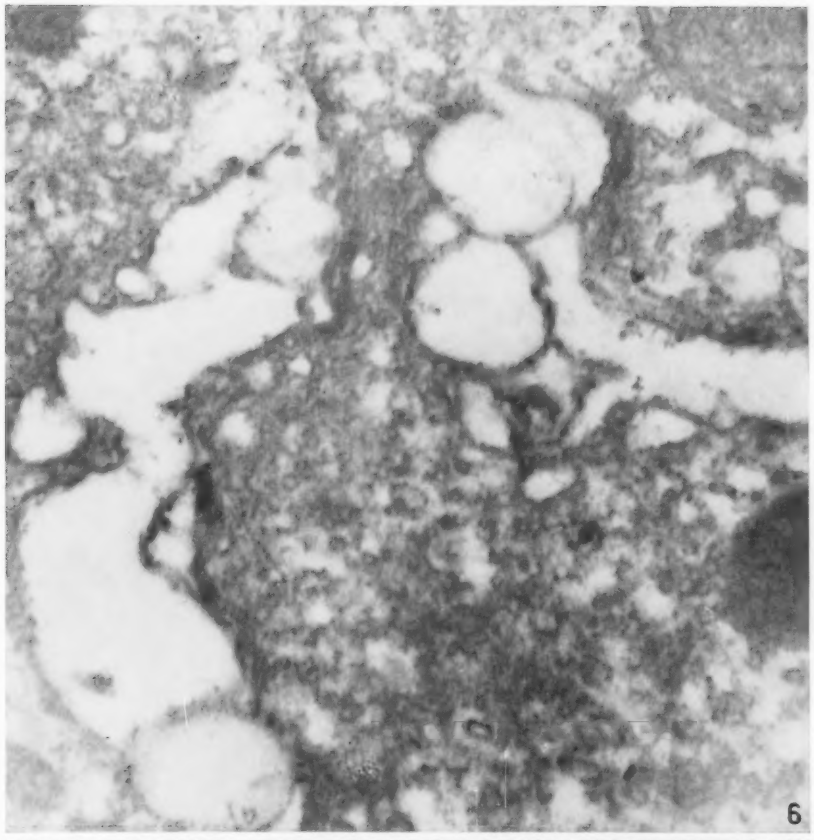

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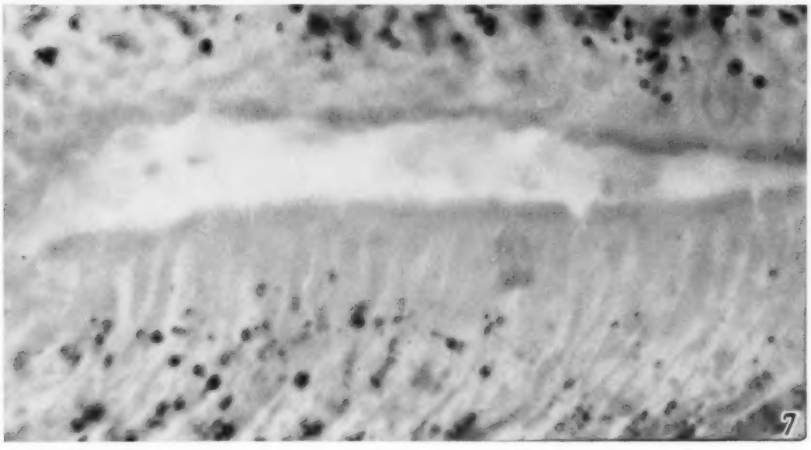

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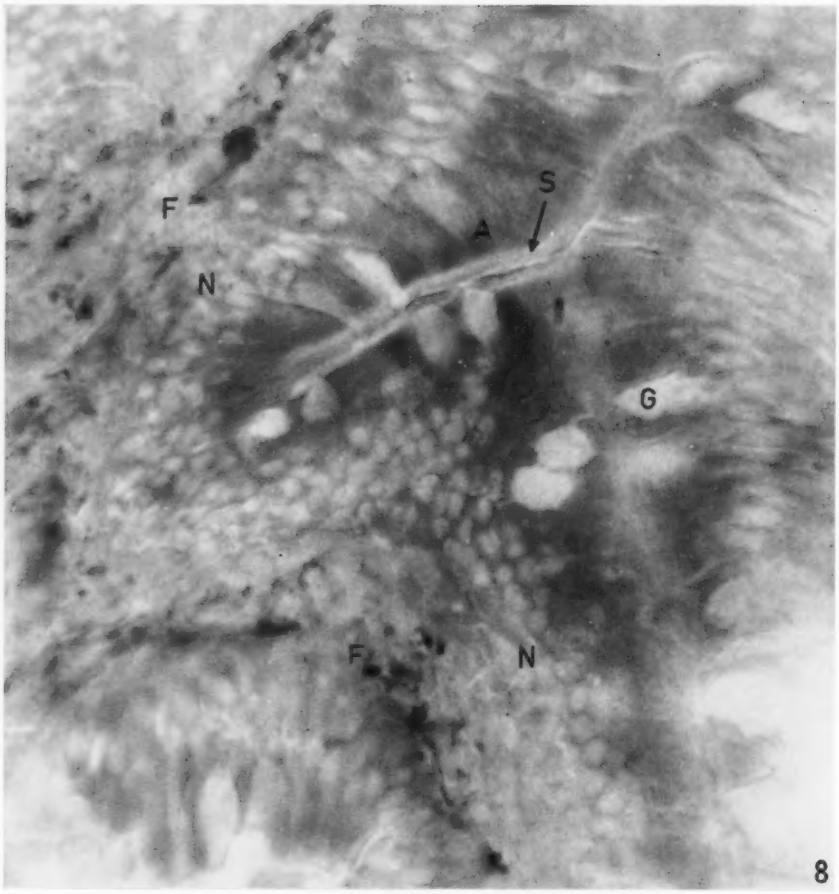

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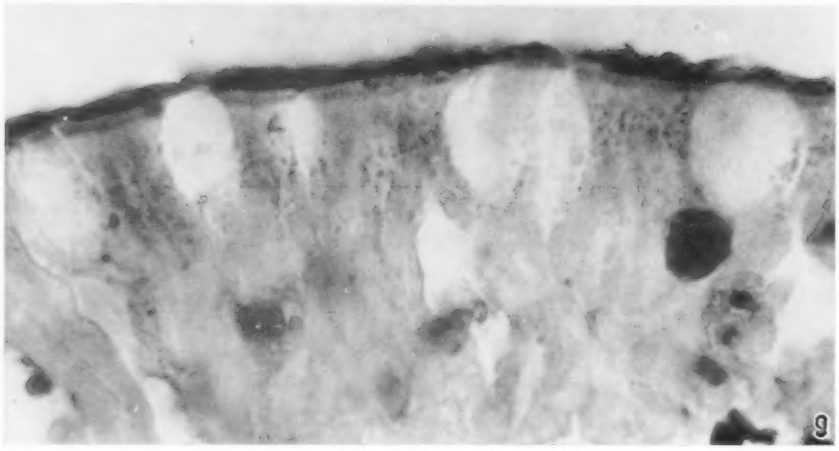

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Mitochondria. Janus green stained mucosal smears as well as the electron micrographs show an abundance of elongated mitochondria in the cell portion between the apical cell pole and the free cell surface, with the exception of a thin mitochondria-free subcuticular zone close below the surface and the juxtanuclear zone, which is fairly poor in mitochondria. The mitochondria are usually oriented along the longitudinal axis of the cell and have the usual lamellated structure. The mitochondria in the apical cell region are often cut in a curved position thus more or less enclosing a vacuole-like structure which has a lower electron density than the surrounding ground substance (Figs. 3, 4, 5).

Golgi zone. The Aoyama impregnations indicate that a large, but not very reticulated, Golgi apparatus is situated in the above-mentioned mitochondria-poor zone close to the distal pole of the columnar cell nucleus. It is interesting to note that the impregnations are generally more successful on the appendices cells than on the intestine cells. In the same region the electron microscope micrographs reveal a well-defined Golgi complex, which consists of numerous large and often widely dilated cisternae and membranes (Fig. 6).

Other cytoplasmic constituents. Some different components can be distinguished in the homogeneous ground substance of the cytoplasm in the form of free granules and small vesicles. Scattered granulated membranes which may be seen in the cytoplasm are apparently parts of the poorly developed endoplasmic reticulum.

Occasionally very large and electron-dense inclusions are found. They are apparently identical with the lipide droplets seen in the light microscope preparations after fixations containing osmic acid (Fig. 7).

Histochemistry of the caecal cells

Carbohydrate reactions. It has not been possible to distinguish any carbohydrate accumulations in the columnar cells, although, as could be expected, the goblet cell secretion gives all the reactions for mucoid substances. WEINREB and BILSTAD (1955) obtained reactions for mucopolysaccharides in the striated border of the trout caecum but this observation could not be confirmed in this case. Glycogen was not found in the preparations.

Protein reactions. Neither the Millon reaction for tyrosine nor the ninhydrin/Schiff technique for α-amino acids has given any indications of protein accumulations in the caecal mucosa. Also the Astra blau method fails but the goblet cell secretion reacts with this method after permanganate oxidation. The pepsin digestion is especially effective on the striated border of the columnar cells.

The methyl green/pyronine procedure shows that the nucleoprotein content of the columnar cells is low, as can be expected in view of the insignificant ergastoplasm shown in the micrographs.

Lipide reactions. After fixation in osmium-containing fixing fluids (Fig. 7) large dark brown inclusions are often abundant in the columnar cells, especially in the proximal caecal portion (cf. GREENE 1913). Numerous inclusions of the same appearance are also found in the stroma of the epithelial folds.

The Sudan black B preparations give very adequate results after the carbowax technique (Fig. 8). The striated border of the columnar cells gives a faint reaction only and the subcuticular zone does not react at all. The apical cytoplasm, the "mitochondria zone", gives on the other hand an intense lipide reaction. Tiny lipide droplets are also often seen in goblet cells. Finally the stroma of the folds contains richly sudanophile granules and droplets of different sizes.

The columnar cell cytoplasm is stained bluish in the Nile blue preparations, especially in the apical zone. The striated border has a grey-blue tone.

Phosphatases. Acid phosphatases occur abundantly in the microvilli of the caecal epithelium cells (Fig. 9). The same preparations also show a number of dark granules in the apical cytoplasm below the subcuticular zone, that is in the "mitochondria-zone". The Ca|Co method for alkaline phosphatases gives a very strong reaction in the fold stroma and indicates an intense phosphatase activity in the epithelium as well.

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Discussion

The caecal mucosa of *Perca* is built up of two types of cells: the goblet cells and the columnar epithelium cells. Occasionally some wandering cells may be seen among these cells.

The goblet cells have the usual appearance and release mucinogen into the caecal lumen. The function of this alkaline mucus is probably here as in other cases to yield mechanical and chemical protection.

The columnar cells show all the morphological characteristics of absorbing cells, when compared to intestinal epithelium cells of *Perca* and the absorbing cells described by other authors from various animals (GRANGER and BAKER 1950, DALTON 1951, WEISS 1955, ZETTERQVIST 1956, CLARK 1959, HARTMAN et al. 1959, HAUBRICH et al. 1959). They are thus provided with microvilli, have an abundance of mitochondria in the apical cell cytoplasm and an elaborate Golgi complex near the apical nucleus pole. They show, on the other hand, no signs of having glandular properties like the goblet cells. Already the appearance of the cell border speaks against this interpretation, furthermore they are rather poor in ergastoplasmic structures and finally no inclusions can be discovered, which may be considered to be secretory material produced by the cells.

There is also histochemical evidence of a resorptive activity of these cells. There are strong indications of lipide resorption. Although the animals had apparently not been killed in a very pronounced state of lipide absorption, the cells

contain numeros lipide granules with about the same size and distribution as those in other lipide-absorbing cells (BAKER 1951, WOTTON and HAIRSTONE 1959). The microvilli probably contain lipase (cf. RAHIMULLAH 1945, AL-HUSSAINI and KHOLY 1954) and absorb the lipides into the cell cytoplasm. The abundance of lipide material among the mitochondria suggests the importance of these structures as well as of the Golgi zone (WEISS 1955) in the lipide metabolism of the cells. Finally the lipides pass the epithelium and are partly accumulated in the stroma in the form of large droplets of neutral fats. These observations are quite in accordance with the suggestion of GREENE (1913) that the chief function of the caeca is fat resorption.

As can be expected no signs of protein or carbohydrate resorption could be found with the methods used here, although it may of course be possible that the abundance of phosphatases is related to phosphorylations of carbohydrates as well as of lipides. It is also possible that a resorption of water and inorganic ions takes place here.

The observations have strongly indicated that the function of the caeca, in *Perca,* is exclusively resorptive and that, as has been suggested before, the caeca are merely a surface enlargement mechanism of the intestine. The renewal of intestine material may seem to be very ineffective in these blind guts (cf. KRUKENBERG 1878), the more so as it is now apparent that their cells totally lack cilia in this species. It must be remembered, however, that the caecal muscle layers are fairly well developed, which indicates the occurrence of peristalsis or, perhaps rather, a pumping action of the caeca, because the longitudinal muscles are proportionally better developed here. Furthermore the mucosal muscle layer and the stroma of the folds, which is very rich in reticular fibres, suggest an effective mucosal motility in the caeca. These facts seem to make an effective passage of food material possible, at least in a species with such short appendages as *Perca.*

Summary

The "pyloric caeca", *appendices pyloricae,* of *Perca fluviatilis* were studied by means of the electron microscope and with some histochemical methods. The caecal mucosa consists of goblet cells with the usual appearance and of tall columnar cells. The latter type is provided with phosphatase-rich microvilli and they have an abundance of mitochondria and well-developed Golgi complexes. A close relationship between some apical mitochondria and vesicle-like structures of low electron density is described. The columnar cells are strongly sudanophile and fat resorption seems to be their main function. Ciliated cells and signs of glandular activity, other than that of the goblet cells, have not been found in the caeca of this species.

Acknowledgments

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Figure captions

Fig. 1. Caecal mucosa fold with columnar cells and some goblet cells. The striated border appears as a dark streak and several connective tissue cell nuclei are seen in the stroma. "Trichrome", 480 ×.

Fig. 2. Basement membrane with terminating columnar cells. In the upper left corner the endoplasmic reticulum of a goblet cell is seen. Below the membrane connective tissue cell processes. 21,000 × (electron optic magnification 6,000 ×).

Fig. 3. Mitochondria in close relationship to vacuoles. 17,000 × (el.opt. 5,000 ×).

Fig. 4. A mitochondrion which almost surrounds a vacuole, which however seems to be situated outside the outer membrane of the mitochondrion. 27,000 × (el.opt. 9,000 ×).

Fig. 5. Apical region of a columnar cell (left) and a young goblet cell (right). Note the disintegrating microvilli of the goblet cell. D. Mucinogen droplet. L. Caecal lumen containing some material. M. Mitochondrion partly enclosing a vacuole-like structure. S. Subcuticular zone. 34,000 × (el.opt. 10,000 ×).

Fig. 6. Golgi apparatus of a columnar cell. Several lamellae and some widely distended cisternae. 43,000 × (el.opt. 12,000 ×).

Fig. 7. Caecal epithelium after osmic acid fixation (Flemming). Several osmiophile inclusions are found, especially in the basal region of the columnar cells, which is best seen in the obliquely cut upper epithelium. Light microscope, 1,000 ×.

Fig. 8. Sudan black B reaction after carbowax embedding technique. A. Apical intensely reactive columnar cell region. F. Fat droplets in the stroma. G. Goblet cell. N. Nuclear zone. Note the lipide-free subcuticular zone, S. 800 ×.

Fig. 9. Acid phosphatase test. The striated border gives an intense reaction and several droplets appear in the apical cytoplasmic zone of the columnar cells. 1,000 ×.

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Cone Types and Cone Arrangement in the Retina of Some Cyprinids

by

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Introduction

The distribution of different kinds of cones in the Cyprinid fishes is very little known, and many contradictory statements can be found in the literature. Thus among 12 Cyprinids WUNDER (1925) did not find any species having double cones, whereas other authors (see below in the description of each species) have found double cones in many of those species. Even triple cones have been found in some Cyprinids: *Leuciscus cephalus, Tinca tinca, Cyprinus carpio* (VRABEC 1955) and *Phoxinus laevis* (LYALL 1956, 1957 b); quadruple cones have been found only in *Phoxinus* (LYALL 1956, 1957 b). There has also been discussion as to whether equal double cones exist within this family or whether only unequal double cones are to be found there. In connection with that question, the possible existence of a regular cone pattern in the retina of the Cyprinids has also been treated. Thus for instance LYALL (1957 b) denies the existence of regular cone patterns in species having only unequal double cones. In the same paper, however,

he figures a regular cone pattern in the peripheral regions of the minnow retina (this pattern, he found, grows looser towards the centre of the eye). MÜLLER (1952) also describes regular rows of double and single cones in this species. MC EWAN (1938) found in *Barbus lineomaculatus* a very diffuse pattern, regarded by her as a form of square pattern of the type that by LYALL (1957b) was found to be the commonest among teleosts. EIGENMANN & SHAFER (1900) also found a cone arrangement in the American Cyprinid *Pimephales notatus*, figured as a kind of square pattern. Owing to this incomplete knowledge and these partially contradictory records of the cone types of the Cyprinids and the cone arrangement in the retina, I have studied these questions in 13 Cyprinids.

Material and methods

Eyes from the following species were examined:

Leuciscus rutilus	9	specimens	(16—250 mm)
Leuciscus erythrophthalmus	3	„	(150—170 mm)
Leuciscus idbarus	4	„	(40—300 mm)
Abramis brama	5	„	(200—300 mm)
Abramis blicca	6	„	(45—220 mm)
Abramis vimba	3	„	(360—400 mm)
Cyprinus carpio	1	„	(170 mm)
Cyprinus carassius	2	„	(350 mm)
Carassius auratus	2	„	(90—140 mm)
Tinca tinca	2	„	(50—280 mm)
Brachydanio rerio	12	„	(9— 30 mm)
Phoxinus phoxinus	15	„	(9— 60 mm)
Alburnus alburnus	11	„	(9— 42 mm)

All the eyes used have been totally light-adapted. The eyes were fixed in Bouin's fluid. Some of them were sectioned undivided in various directions. From other eyes, 5—15 small pieces of the retina (about 2—3 mm^2 in size) were cut out and their origin mapped. They were cut tangentially and transversally in 4—8 μ sections, which were stained in Heidenhain's Azan—Mallory, Regaud's Haematoxylin and Foot's modification of Masson's trichrome staining (FOOT 1933).

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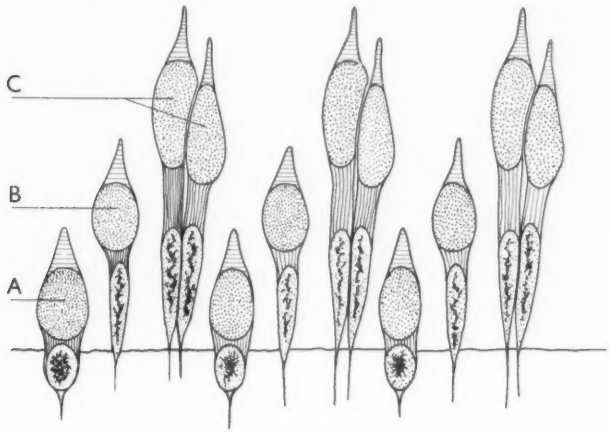

Fig. 1. Diagram of the cone types of *Leuciscus rutilus* as seen in transverse section of the retina of young stages. The ellipsoids are found in three separate levels, each containing those of one cone type. A: short single cone, B: long single cone, C: unequal double cone.

Results

Leuciscus rutilus

HANNOVER (1843 and 1844), examining fresh retinas of the roach, found both single cones and double cones. However, he did not distinguish between unequal and equal double cones, but describes all double cone elements as twin cones —"tvillingtapper" (1843) or "cônes jumeaux" (1844). GREEFF (1900), giving a more detailed description of the visual cells of the roach, describes four kinds of cones: 1. Great single cones with incomplete outer segment. 2. Slender single cones. 3. Small single cones. 4. Unequal double cones. LYALL (1957 b) also records the presence of unequal double cones in this species.

In my own material I have found three clearly separated types of cones: 1. Short single cones, without myoid. 2. Long single cones. 3. Unequal double cones. In transverse sections through the retina of young stages, these three cone types are very easily distinguished. The nuclei of the short single cones in these stages lie inside the external limiting membrane, and the ellipsoids of the cones are found in three clearly visible levels (Fig. 1). This organization of the cones is the same as that found by MÜLLER (1952) in *Lebistes*, where he classifies the three kinds of cones according to their length. In more adult roaches, however, I have found this organization to be more diffuse, the long and slender single cones showing a variation in length between the original length and one corresponding to that of the double cones. I have not found any cone type corresponding to the great single cone described by GREEFF. In addition to these three

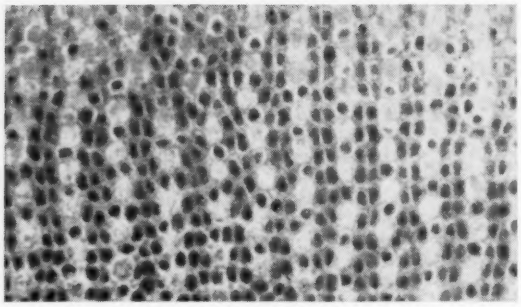

Fig. 2. Tangential section of the retina of *Leuciscus rutilus*, 22 mm, showing the cone arrangement. Azan-Mallory, 6 μ, 800 ×.

main types of cones, triple cones, most suitably studied in tangential sections, occur in the more central regions of the eye.

In tangential sections one can also see that only the cone types mentioned above occur in this species, and that the single and double cones are organized in a regular cone pattern. This arrangement of the cones in the roach retina has not been discussed earlier, apart from LYALL's (1957 b) indication of there being no definite cone pattern in this species. A tangential section through the eyes of young stages, however, shows a very regular cone arrangement, in which rows of double cones alternate with rows of single cones (Fig. 2). In the latter, long and short single cones regularly alternate. The rows follow the direction of the nerve fibres of the innermost layer of the retina, i.e. mainly the same direction as the radii of the eye. The same pattern will also be found in adult specimens (Fig. 3 a). In this adult pattern one can also see a regular alternation of the direction of the double cones. This can be seen owing to the fact that in the double cone, one half partially displaces the other, the one side of which in this manner becomes concave. In a tangential section, the two halves of an unequal double cone also seem to have different staining properties, which has also been observed by MÜLLER (1952) and LYALL (1957 b). However, it should not be taken for granted that this difference in colour depends upon different staining properties. As can be seen from Fig. 8 A, the ellipsoids of the two halves of the double cones of this species are unequal in form and do not stain homogeneously. Thus a tangential section, cut through the light part of one half, can pass through the dark part of the other. These differences between the two halves make it possible, in a tangential section, to state that alternate double cones in the rows have the same direction.

The cone arrangement is thus found to be the same as the peripheral one in the retinas of *Lebistes* (MÜLLER 1952) and *Phoxinus* (LYALL 1957 b), and can be generalized as shown in Fig. 4. LYALL also finds that this pattern grows more diffuse towards the centre of the eye, and that it is totally dissolved in the central regions of the eye. The same observation can be made in the roach, but the pattern does not change in the same manner in all the regions of the eye. In tangen-

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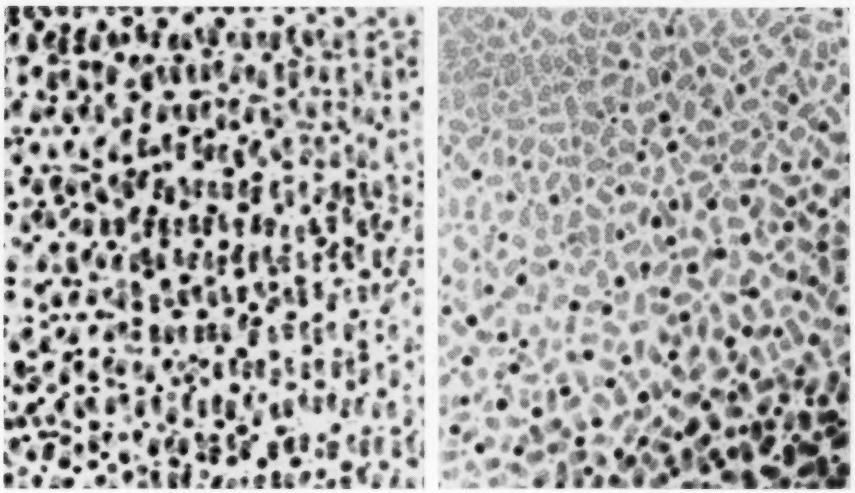

A B

Fig. 3. Tangential sections of the retina of ad. *Leuciscus rutilus* (250 mm), showing the difference between the cone arrangement in different parts of the eye. A, from the equator of the eye, 5 mm temporal to the optic nerve. B, the same retina, 5 mm ventral to the optic nerve. Azan-Mallory, 7 μ, 200 $\times$.

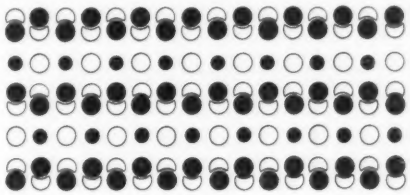

Fig. 4. Diagram of the generalized cone pattern of *Leuciscus rutilus*.

tial sections from different regions of the eye, one can see that the pattern is well preserved along the horizontal equator of the eye (Fig. 3 A), but is totally dissolved in the dorsal and ventral parts (Fig. 3 B).

This variation of the pattern in different regions of the eye is shown in diagrams in Fig. 5. The diagrams are obtained by measuring the angle formed by the long diameter of the double cones and the radius. In the ideal pattern, this angle should be 90°, as will be seen in Fig. 4. A great number of such angles are measured from photographs and in a Reichert projection microscope ("Lanameter"). The deviations from the ideal position are classified into groups of 5°, and the percentage falling into each group is plotted in the diagram. Thus a diagram showing a strongly pronounced concentration towards the middle (as Fig. 5 A), will represent a pattern with small deviations from the ideal pattern, whereas a diagram without such a concentration (Fig. 5 B) represents an area

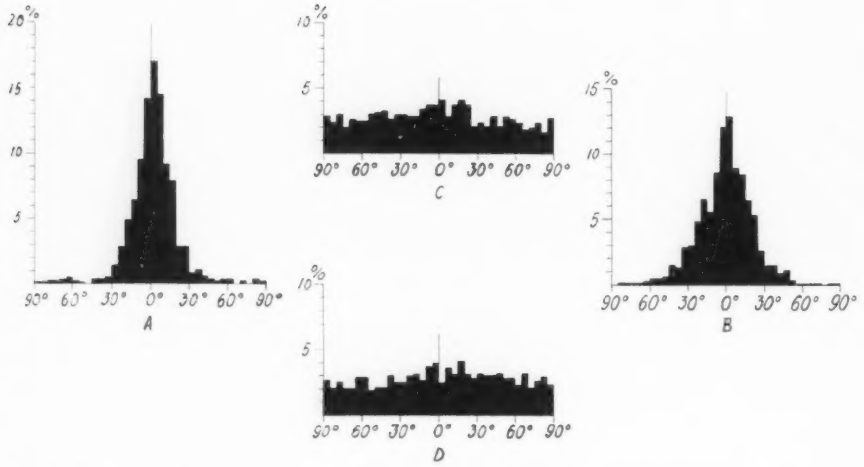

Fig. 5. Diagrams of the variations in the double cone arrangement in the retina of *Leuciscus rutilus*, obtained as described in the text. Measurements from the right eyes of two specimens of equal size (250 mm) plotted together in the diagrams. A, temporal part of equator (1,052 double cones measured), B, nasal part of equator (1,506 double cones), C, dorsal (1,096 double cones) and D, ventral part of the retina (1,579 double cones). All measurements taken at 4 mm from the optic nerve.

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of the eye where no pattern can be found. Thus the diagrams, Fig. 5, will show the difference between the equator and the other parts of the eye mentioned above. It can also be seen, here as well as in the sections, that the most exact pattern is to be found in the posterior (temporal) part of the equator.

Leuciscus erythrophthalmus

The cone types of the rudd have been very little discussed. HANNOVER (1843) found that the cones of the rudd were of the same types as those of the roach, and FRIIS (1879) describes both single cones and "twin cones" ("tvillingetapper"). But as FRIIS does not distinguish between unequal and equal double cones but even sometimes figures unequal double cones as identical twin cones, his mention of the double cone elements is of no significance. VERRIER (1928) also found double cones in this species, but does not distinguish between the different kinds of double cones, naming all "cônes doubles". The cone arrangement has only been mentioned by LYALL (1957 b), who states that there is no regular pattern in this species. I have found that the cones of this species are mainly of the same types as those of the roach, but they are a little more bulky (cf. Fig. 8 B). Double cones and both short and long single cones are present. The variation in length of the long single cones is more pronounced than in the roach, and therefore the limit between the two kinds of single cones is not so sharp. This

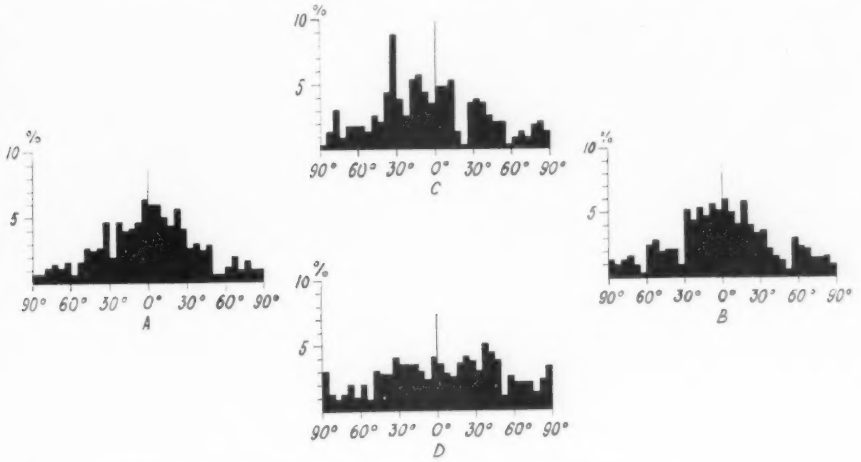

Fig. 6. Diagrams of the variations in the double cone arrangement in the retina of *Leuciscus erythrophtalmus*, obtained as the diagrams in Fig. 5. Measurements 4 mm from the optic nerve. A, 464, B, 407, C, 226, D, 424 double cones.

can also be seen in the cone pattern, which is not nearly as regular as that of the roach. In the more peripheral parts of the eye one can, it is true, trace the same pattern as that shown in Fig. 4, but in the more central parts it is very loosely arranged. The rows of double cones are diffuse and the single cone rows—if distinguishable—do not show the same regular alternation between short and long single cones. In the diagram, Fig. 6, one can see that the pattern is most dissolved in the ventral part of the eye. In the dorsal part the original position is a little better retained, and, as in the roach, the most exact pattern is found along the equator.

Triple cones are seen in most parts of the eye, but the greatest number was seen in the centre of the eye.

In the rudd I have also found a kind of visual element, mostly resembling a small, slender, slightly stained single cone without myoid and with its nucleus lying, at least mainly, inside the external limiting membrane. These elements may perhaps be the same as those described by LYALL (1957 b) in *Phoxinus* and which are assumed by him to be intermediate stages in a supposed transmutation of small single cones to rods.

Leuciscus idbarus

The only description of the cone types of the ide seems to be that of FRIIS (1879). He found both "twin cones" and single cones but, as mentioned above, he does not distinguish between unequal and equal double cones, and he has

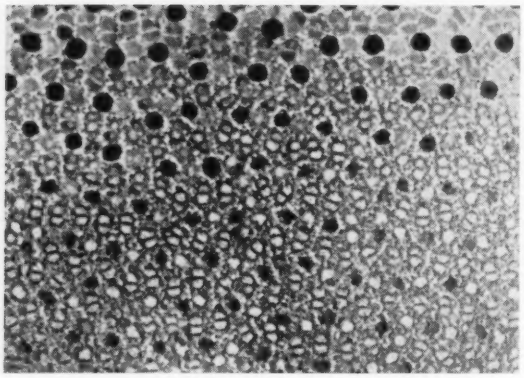

Fig. 7. Tangential section of the retina of *Abramis brama* (200 mm), taken from the temporal part of the equator of the eye, 4 mm from the optic nerve. Azan-Mallory, 7 μ, 300 ×.

not figured the cones of this species. I have found that the cones of the ide are very similar to those of the roach. In young stages the same cone types will be found as has been figured in the roach (Fig. 1). In the adult specimens the limits between the cone types were more diffuse. I have also found triple cones as well as the "intermediate elements" described above in the rudd.

The cones are arranged in a pattern similar to that of the roach; not as exact as in this species, but more definite then that of the rudd. As in these species, the pattern is clearly recognizable only in the more peripheral parts of the retina. In the central regions it is almost totally dissolved, except along the equator of the eye, where it will easily be found even quite near the centre of the eye. The pattern is most regular in the temporal parts of the equator.

Abramis brama

The cones of the bream were first described by HANNOVER (1843) as single cones and twin cones. FRIIS (1879) found single as well as double cone elements, and figured the latter as equal double cones. KÜHNE & SEWALL (1880) also found both kinds of cones, the double ones being only named "Doppelzapfen". However, by studying their drawings, one can clearly see that the cones are unequal double cones. WUNDER (1925), examining the visual cells of a number of fish species, found only single cones in the bream.

In my own material I have found both single and double cones, all very similar to those of the roach described above. Thus the single cones are of two kinds—short and long—and the double cones are unequal ones (Fig. 8 C). Triple cones have also been observed in the central parts of the retina.

The arrangement of the cones is the same as that of the roach. I have found the clear arrangement in distinct rows of regularly alternating cones (Fig. 7) and the same variation in the exactness of the pattern in the different regions of the retina.

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Abramis blicca

In this species, too, HANNOVER (1843) found "twin cones". WUNDER (1925), however, only describes single cones. I have observed single and double cones, all very like those of the bream (Fig. 8 D). Triple cones are frequent even in this species in the central parts of the retina, especially ventral to the optic nerve. The cone pattern is the same as that described above, and shows the same degree of exactness and variations between the different regions of the retina as in the roach and the bream.

Abramis vimba

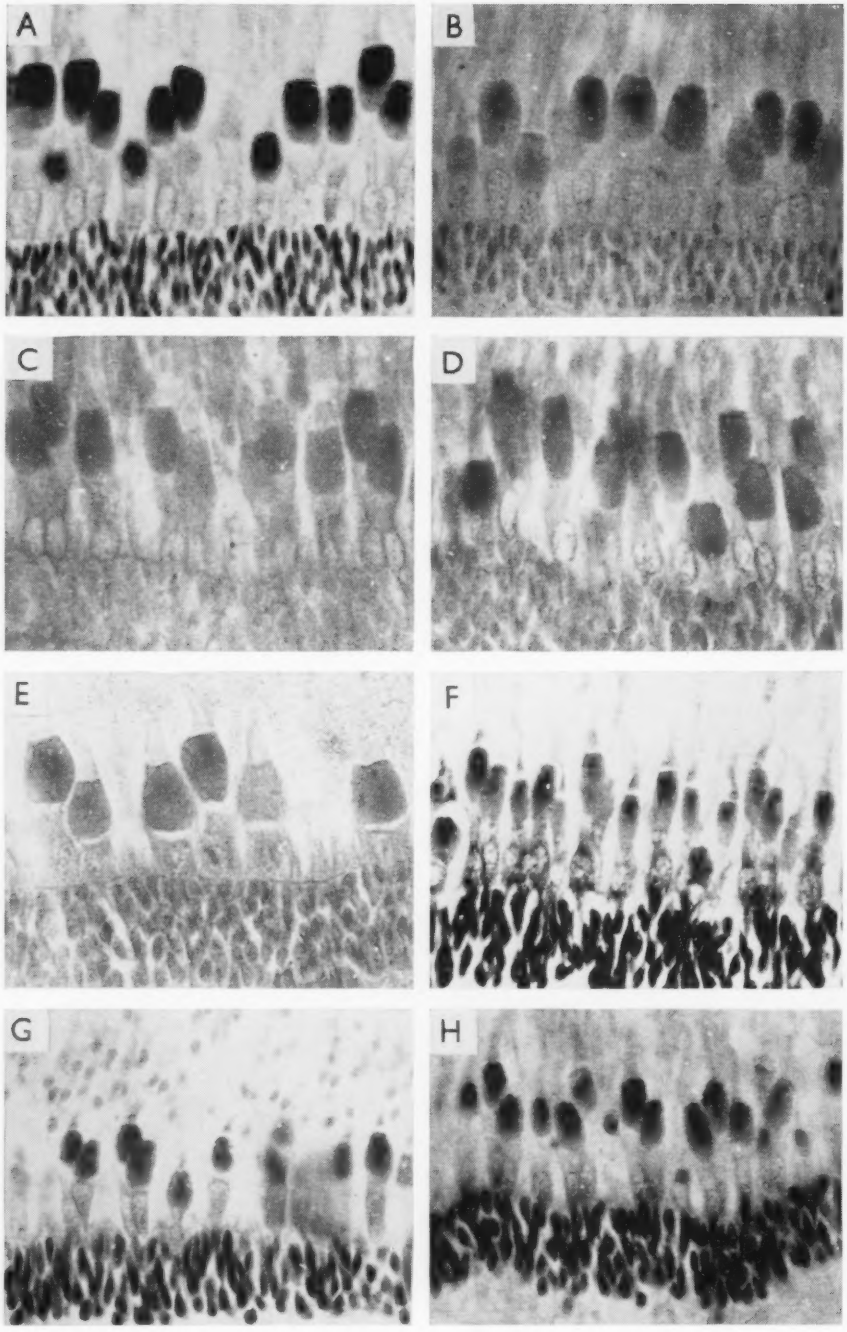
The cones presented here are of the same type as in all the previous species, i.e. unequal double cones and the two kinds of single cones, but they are a little more bulky, as seen from the photograph, Fig. 8 E. Further, triple cones are common, especially in the central regions, and I have also observed the "intermediate elements". The cones are arranged in rows as in all the species described earlier, and the most exact pattern is also seen in the temporal part of the eye, on and near the equator. However, the normal position of the double cones in this species does not seem to be at right angles to the radii, but at an angle of about 70—80°, as is shown in Fig. 10.

In the more central parts of the fundus, especially ventral to the optic nerve, I have observed a structure difficult to understand. It consists of groups of cones, in their shape most resembling small single cones, each group comprising about 10—15 cones. These cones are tightly clustered, and only a few rods may pass between them. The rod nuclei inside such a group also seem quite degenerate (cf. Fig. 10 B). I have noted these groups in the three specimens I have examined (all caught in the same locality), but considering the restricted material it is doubtful whether they are specific in this species or are merely an abnormity.

Cyprinus carpio

SCHULTZE (1867) states that *"Cyprinus"* has equal double cones, and LYALL (1957 B) is also of this opinion. VERRIER (1928), not distinguishing between unequal and equal double cones, only records the presence of double cone elements ("cônes doubles"). WUNDER (1925) did not discover any double cone elements, only single cones. Triple cones are described by VRABEC (1955).

I have found that single, double and triple cones are all present. The single cones are, in principle, of the different types described in all the species above, but they vary in shape and length more than in those species. They can be clearly separated only through the absence of the myoid in the short single cones. The double cones are of the unequal type (Fig. 8 F), but I have found a few double



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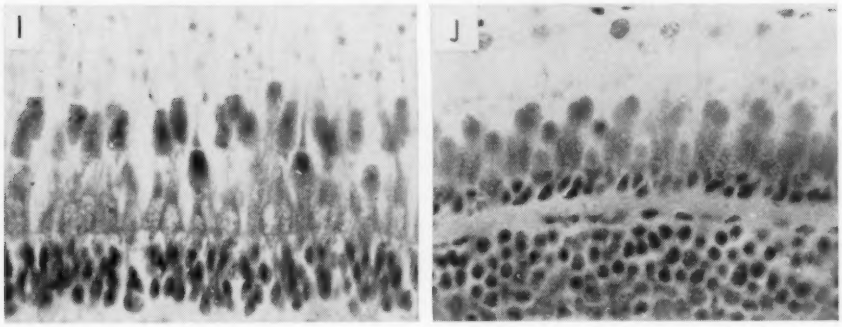


Fig. 8. Transverse sections of the retinas of some Cyprinid species, showing the appearance of the cone types described in the text. A, *Leuciscus rutilus,* Azan-Mallory, 7 μ. B, *Leuciscus erythrophthalmus,* Azan-Mallory, 7 μ. C, *Abramis brama,* Azan-Mallory, 7 μ. D, *Abramis blicca,* Azan-Mallory, 7 μ. E, *Abramis vimba,* Azan-Mallory, 7 μ. F, *Cyprinus carpio,* Foot-Masson, μ. G, *Cyprinus carassius,* Foot-Masson, μ. H, *Carassius auratus,* Foot-Masson, μ. I, *Tinca* tinca, Azan-Mallory, μ. J, *Brachydanio rerio,* Foot-Masson, μ. All Figs. 700 ×.

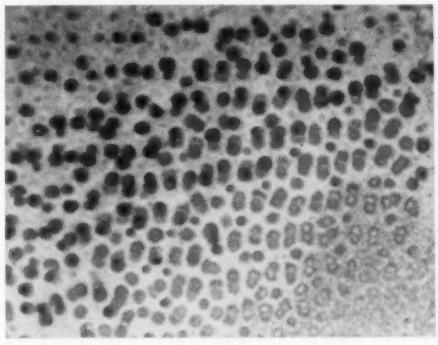
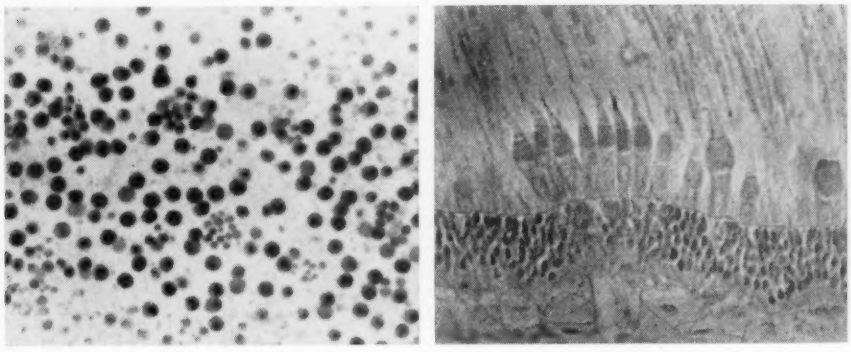


Fig. 9. Tangential section of the temporal part of the retina of *Abramis vimba,* 4 mm from the optic nerve. Azan-Mallory, 7 μ, 150 ×.



A B

Fig. 10. A. Tangential section of the retina of *Abramis vimba,* 3 mm ventral to the optic nerve, showing the tightly clustered groups of cones mentioned in the text. Azan-Mallory, 7 μ, 200 ×. B. Transverse section of one such group. Azan-Mallory, 6 μ, 400 ×.

cones with quite equal halves. The cone arrangement is seen to be very diffuse, and a clear pattern of the type described above does not to exist in this species.

Cyprinus carassius

HANNOVER (1843) and FRIIS (1879) describe in this species single cones as well as "twin cones", while KRAUSE (1886) found unequal double cones. WUNDER (1925) only found single cones. I have found the double cones to be of the unequal type (Fig. 8 G); the single cones are of the same two kinds as has been described above, even if they vary a little in length and shape. Triple cones have been observed.

The cone arrangement is, in principle, of the type shown in Fig. 4. However, the direction of the long diameter of the double cones changes more than in the roach and the bream for instance, and somewhere in the rows a clear tendency to a zigzag pattern can be seen, in which the double cones tend to be at an angle of 45° to the direction of the rows (cf. Fig. 11). In the dorsal and ventral parts of the eye, this pattern is very loosely arranged, but along the equator of the eye distinct rows can be seen, following the radii.

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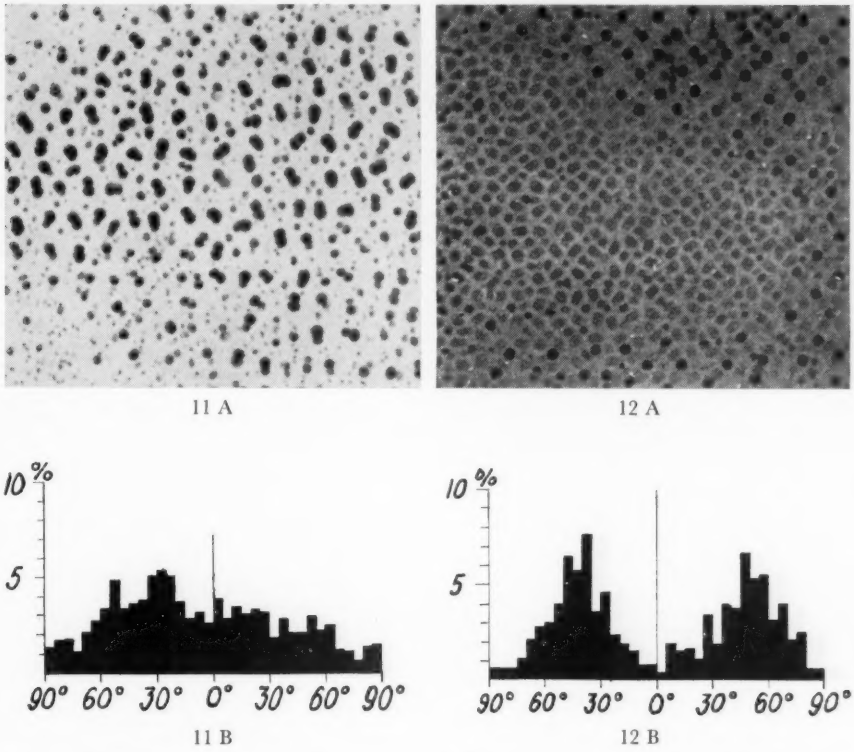
Carassius auratus

Double cone elements, without further specification, are described by NUNNELEY (1858) and VERRIER (1928). WALLS (1942 p. 586) states that the species has unequal double cones. I have found both unequal double cones and the two kinds of single cones, which are all very similar to those of *Cyprinus carassius* (Fig. 8 H). A quite distinct pattern can be seen in this species, being most elaborate in the equator of the eye, but difficult to distinguish in the dorsal and ventral regions of the retina. However, the pattern of this species apparently differs considerably from the pattern common among the species above. In the goldfish, I have found a quite regular orientation of the long diameter of the double cones into an angle of about 45° to the direction of the rows (Fig. 12). In this manner the pattern becomes very similar to the "square pattern" found by LYALL (1957 a, b) to be the most common pattern among teleosts. But it is not as exact and regular as in the trout and the perch for instance. Only in one of my two goldfishes was this pattern as clear as is shown in the photo; in the other it was very diffuse and not clearly recognizable. This may perhaps be due to the great variation in morphological characters found in this species, which has been domesticated and cultivated for such a long time.

Tinca tinca

HANNOVER (1843) and FRIIS (1879) both describe single cones and twin cones in this species, while WUNDER (1925) even in this species found only single cones.



11 A 12 A

11 B 12 B

Fig. 11. A. Tangential section of the retina of *Cyprinus carassius*, temporal to the optic nerve. Foot-Masson, 6 μ, 700 ×. B. Diagram of the directions of the double cones in the area photographed in A. 940 measurements.

Fig. 12 A. Tangential section of the retina of *Carassius auratus*, temporal to the optic nerve. Foot-Masson, 6 μ, 700 ×. B. Diagram of the directions of the double cones in the same area, 476 measurements. A clear tendency to a zigzag pattern is seen both in the photograph and in the diagram.

VRABEC (1955) does not discuss the presence of single or double cones, but records the existence of triple cones even in this species. I have found the cones of the tench to be, in principle, of the same shape as most of the cones described above (see Fig. 8 I). The single cones are relatively distinctly separated into long and short ones, and the great variation in length found especially in *Cyprinus carpio* is not found in the tench. Triple cones are also seen. Most of the cone nuclei are more or less sunk into the external limiting membrane, but not as deeply as in the goldfish. No nuclei are found to be more than half-way inside the membrane.

The arrangement of the cones as seen in tangential sections is not as exact as in many of the species described above. In the dorsal and ventral parts of the eye

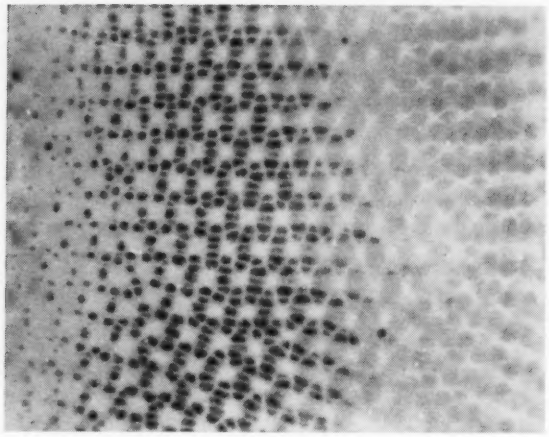


Fig. 13. Tangential section of the retina of *Brachydanio rerio*, showing the cone pattern and the arrangement of the cones in rows corresponding to the radii of the eye. Foot-Masson, 4 μ, 700 $\times$.

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no definite pattern can be recognized, but in the equator quite distinct rows can be seen. The arrangement of the cones within the rows, however, is not very regular, and the typical pattern figured in Fig. 4 cannot always be distinguished without difficulty.

Brachydanio rerio

This East Indian Cyprinid, common in aquaria, is also found to have, in principle, the same characteristics of the cones and their arrangement as the preceding Cyprinids. The three cone types are even in the adult very distinctly separated into different levels (Fig. 8 J), as found in young roach (cf. Fig. 1 p. 279). The nuclei of the short single cones are bulky and situated inside the external limiting membrane, the other nuclei being long and slender, and outside the membrane. The unequal double cones have relatively small ellipsoids.

In tangential sections taken just outside the external limiting membrane, the cone pattern looks a little strange at the first glance (cf. Fig. 13). It is possible, however, to distinguish the rows of double cone nuclei, regularly alternating with rows of single cones. The bulky ellipsoids of the short single cones, situated just outside the external limiting membrane, displace however the alternate double cone nuclei, so that the rows of these nuclei will look a little irregular or zigzag. On a more external level the great ellipsoids of the long single cones will have the same effect. Still more externally one can find the regular rows of double cones, regularly changing their directions. This very exact and regular pattern I have found in all parts of the eye, and no special exactness localized to the equator has been traced.

Cone Types and Cone Arrangement in the Retina

Phoxinus phoxinus

The cones of the minnow are quite well known. v. FRISCH (1925), in his experiments on the light and dark adaptation of the minnow, found two different kinds of cones: those with myoid and those without myoid. The latter are figured with their nuclei inside the external limiting membrane, and may be considered to be the short single cones. He does not mention anything about double cones. WUNDER (1925), it is true, did not find any double cones, but he has instead described a series of single cone types, which he considers to be different stages of cone development. This is disclaimed by MÜLLER (1952), who gives a detailed description of the different cone types, which are found to be unequal double cones and two kinds of single cones. He also finds a difference in staining properties between the two halves of the double cones, and between the different cone types. LYALL (1957 B) has also studied the cones and cone pattern of the minnow, and found the same types as MÜLLER. Further, he found both triple and quadruple cones.

In my material I have also found the cone types mentioned, very clearly separated in both tangential and transverse sections, and organized in levels in the same manner as in young roach (cf. Fig. 1). The arrangement of the cones has been studied from tangential sections by MÜLLER (1952), who found regular rows of double and single cones, the double cones forming a zigzag pattern along the rows. LYALL (1957 b) has studied the pattern more in detail, and has found in the peripheral regions the regular pattern with rows of single and double cones, corresponding to the "ideal pattern" figured in Fig. 4. This pattern will change and grow more diffuse towards the centre of the retina. I have found the same conditions in my own material, both concerning the cone types and the cone arrangement.

Alburnus alburnus

I have found nothing in the literature about this species. In a material of 11 young stages (9—42 mm) I have found this species to agree with the last-mentioned species. The three cone types—short single cones, long single cones and unequal double cones—are very easily distinguishable from each other, and are organized in levels as has been figured in Fig. 1. The cone nuclei are found outside the external limiting membrane, with the exception of those of the short single cones, the nuclei of which penetrate the membrane totally or largely. In the young specimens examined the cones are organized in a regular pattern, in which the three cone types alternate as in the ideal pattern (Fig. 4).

Other Cyprinid fishes treated in the literature

In addition to the species mentioned above, some notes on other Cyprinid fishes can be found in the literature. Thus in *Barbus lineomaculatus* McEwan (1938) found both single and double cones. She does not clearly distinguish between equal and unequal double cones, but her drawings and descriptions show that the double cones are of the unequal type. She has also figured the cone arrangement and interprets this as "apparently somewhat similar" to the square pattern found in *Salmo* for instance. However, in her drawing of a tangential section through the retina, no definite square pattern can be seen, but only loosely arranged rows of double and single cones, which sometimes form a square-like pattern and sometimes do not. It therefore most resembles the more diffuse parts of the goldfish eye.

In the American Cyprinid *Pimephales notatus,* Eigenmann & Shafer (1900) found single and double cones, but the character of the double cones is not mentioned. They have also found a cone arrangement which they describe as a form of square pattern.

In *Leuciscus albiensis* Wunder (1925) has seen only single cones. Vrabec (1955), however, found single cones as well as unequal double cones and triple cones. Even in *Rhodeus amarus* Wunder found only single cones. In this species Verrier (1928) found double cones, not described in detail. Hannover (1843) describes the double cones in *Aspius aspius* under the name of "twin cones" but, as mentioned earlier, Hannover did not distinguish between unequal and equal double cones.

In *Gobio fluviatilis* v. Frisch (1925) has performed experiments on the reactions of the cones during the dark adaptation process, but he does not distinguish between different types of cones. As in Phoxinus, cones without myoids are seen in his photographs, probably corresponding to the small single cones. In the same species and in *Leucaspius delineatus* Wunder (1925) found only single cones. As noted above, he has not found the double cones in any of the described in this paper, and therefore his negative results in these species are of no value. Hesse (1904) and Menner (1929) have figured the cones of *Chondrostoma nasus,* but they do not mention any observation of double cones, perhaps owing to the same imperfection of the method as that of Wunder (1925). In their drawings, however, both long and short single cones can be seen.

Discussion

As stated earlier in this paper, there have been different opinions on the distribution of the different cone types and definite cone patterns within the family Cyprinidae. To a large extent the different opinions are attributable to the use of

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unsatisfactory methods. WUNDER (1925), who is often quoted in the literature dealing with these questions, did not, as has been mentioned, find double cones in any one of the Cyprinids which have later been found to have such cones. This will be explained by the method used by him. He has only sectioned eyes of very young specimens and has only studied sagittal sections taken through the centre of the eye. As has been shown several times, the cone pattern in the young Cyprinids consists of regular rows following the radii of the eye, and with the long diameter of the double cones at an angle of 90° to the rows. A sagittal section through the centre of the eye will therefore fall along one such row and will thus divide all the double cones across their long diameter. Furthermore, WUNDER used very thin sections (3 μ), which further diminished the chances of seeing the double cones.

The only sure method of determining the presence of double cones is the use of tangential sections, which was already pointed out by FRIIS (1879). Through a combined use of tangential and transverse sections, one can state that in all the Cyprinids examined, three fundamental cone types exist: short single cones, long single cones and unequal double cones. In addition triple cones exist in many species, and sometimes also quadruple cones.

The existence of regular cone patterns within this family has been discussed by LYALL (1957 b), who states that no definite cone pattern exists in species having unequal double cones, exemplifying this with *Leuciscus rutilus* and *Leuciscus erythrophthalmus*. In this paper it has been shown that a very regular pattern may exist even in many of these species, all having unequal double cones. The patterns formed in all these species can be traced back to the "ideal pattern" (Fig. 4). In some species this pattern is very exact in all parts of the eye *(Brachydanio rerio)*. In other species the original pattern is found only in the equator of the eye, and is very loosely organized in the other parts of the eye (e.g. *Leuciscus rutilus*), in still other species the pattern is very diffuse or not at all recognizable all over the eye (e.g. the carp). The square-like pattern of *Carassius auratus* is not as fundamentally different from the standard Cyprinid pattern as might be supposed at the first glance. MÜLLER (1952) and LYALL (1957 b) have studied the development of this square pattern during the growth of the retina, and they have found that the pattern at the edge of the retina begins in the form of rows of the type shown in Fig. 4. Then the double cones change their direction in the rows, and having changed to an angle of 45°, they form the zigzag rows of double cones making up the square pattern. The tendencies to form a square pattern found in some Cyprinids, do not therefore necessarily mean that these species have a divergent type of cone pattern, but only that it is a little more developed. Thus one can state that in all the species of Cyprinids examined, the same fundamental pattern exists, only varying as regards the degree of development. The statements found in the literature to the effect that a pattern does not exist in many of these species, can be based upon observations made on sections from the

ventral or dorsal parts of the eyes, where the pattern can be absent or very difficult to recognize.

Another problem, discussed in this connection, is the possible correlation of the distribution of equal and unequal double cones with the phylogeny of the fish. Some authors, e.g. WALLS (1942), consider that this correlation exists, whereas LYALL (1957 b) denies such a correlation. He does so referring mainly to the Cyprinids, where, according to him, unequal as well as equal double cones exist, the latter in *Cyprinus carpio*. As has been shown above, this argument is not based upon correct facts, a carp having unequal double cones. Instead of a variation in the type of double cones, I have found the double cones of all the Cyprinids examined, and the other cone types too, to be of fundamentally the same types, varying only within narrow limits.

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Summary

The cone types and cone arrangements of 13 Cyprinids have been studied by means of transverse and tangential sections. In all the species the following fundamental types of cones were found: 1. Short single cones, without contractile myoid and often penetrating the external limiting membrane. 2. Long single cones with contractile myoid. 3. Unequal double cones. 4. In most of the species triple cones have been observed.

In most of the species the three first-mentioned types of cones build up a regular pattern consisting of rows mainly following the direction of the radii of the eye. Rows containing double cones alternate regularly with rows containing only single cones, where the two types of single cones also alternate. This pattern is found to be most perfectly developed in the equator of the eye and has more or less disappeared in the ventral and dorsal parts. In some species (e.g. the carp) the pattern has almost entirely vanished, while in other species, e.g. *Carassius auratus*, it has changed and become more similar to the square pattern known from many teleost fishes. In spite of these differences, the characteristics in common mentioned above indicate the presence of phylogenetically determined cone types, arranged in definite patterns. These vary, it is true, between the different species, but they can all be traced back to a fundamental pattern, common to the whole family.

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The Chondrocranium of Tropiocolotes tripolitanus

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Introduction

The chondrocranium of Lacertilia has been described by several investigators, including the present author. However, certain features in the lacertilian chondrocranium still remain obscure. The present paper deals with the chondrocranium of an Egyptian lizard which is *Tropiocolotes tripolitanus*. An exhaustive description will not be mentioned here, only a review of the important features will be given. Two stages of Tropiocolotes are described, a moderate stage (stage I) and a late one possessing the fully formed chondrocranium (stage II).

Tropiocolotes tripolitanus is an extremely small lizard, belonging to family Geckonidae. Like other geckos, it can live very well and lay eggs very easily in captivity. Its eggs usually hatch in the normal atmosphere without special incubation. The average number of days of the period of incubation for this gecko is 63 days, a rather long period in comparison with other geckos. Each female lays only one egg in every season.

The chondrocranium is studied by the usual method of serial transverse sections and graphic reconstructions made from them. The embryos are stained in toto with Borax carmine and, after sectioning, the sections are counterstained with Picroindigo-carmine.

I am greatly indebted to Prof. Dr. M. R. El-Toubi, Professor of Comparative Anatomy, Faculty of Science, Cairo University for the valuable suggestions and great help he offered me while doing this work.

Stage I

(age: 29 days, total body length: 15.2 mm., Figs. 1—4)

The nasal septum (S.N.) is unfenestrated. Anteriorly the septum is rather low, it gradually increases in height in the posterior direction. Then the dorsal edge of the septum is suddenly lowered, and consequently the septum becomes low in the posterior region of the nasal capsule (Fig. 3). At the anterior end of the nasal capsule the rudiments of the two cupolae anteriores can be observed. Each cupola is perforated ventro-medially by the foramen apicale (Figs. 2 & 3, F.A.), through which the ramus medialis of the ethmoid nerve passes. The parietotectal cartilages (PT.C.) grow out from the dorsal edge of the nasal septum on both sides. No fenestra superior can be observed in the parietotectal cartilage. The rudiment of the lamina transversalis anterior (Figs. 2 & 3, L.T.A.), which is continuous with the ventral edge of the nasal septum, is chondrified.

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Lateral to the parietotectal cartilage, the rudiment of the paranasal cartilage (PN.) is observed. At the present stage the paranasal cartilage is incomplete being perforated by a large gap. This gap separates the dorsal part of the paranasal cartilage from the ventral part of the same cartilage. The paranasal cartilage is completely fused with the parietotectal cartilage and the fusion takes place in a special manner. The ventral edge of the parietotectal cartilage is separated from the rest of the same cartilage and is fused with the ventral part of the paranasal cartilage. Similarly the rest of the parietotectal cartilage is fused with the dorsal part of the paranasal cartilage. Thus there are two cartilaginous plates, one occupying a dorsal position to the other. Each plate is composed of a part of the parietotectal cartilage and a part of the paranasal cartilage without any line of demarcation between the two elements. The dorsal plate, which is composed of the dorsal part of the parietotectal cartilage plus the dorsal part of the paranasal cartilage (Figs. 1 & 3, D.PT.+D.PN.), forms the dorso-lateral wall of this region of the nasal capsule. The ventral one, which is composed of the ventral part of the parietotectal cartilage plus the ventral part of the paranasal cartilage (Figs. 2 & 3, V.PT.+V.PN.), forms a part of the wall of the future concha nasalis. A similar manner of fusion between the parietotectal and paranasal cartilages is observed in *Ptyodactylus* (El-Toubi and Kamal, in press). While the ventral plate ends freely, the dorsal one is continuous posteriorly with two elements: the sphenethmoid commissure (C.S.) and planum antorbitale (P.AN.). Although the dorsal plate is formed of the fused parietotectal and paranasal cartilages without any line of demarcation, yet by taking in consideration the topographical relations, it can be stated, with a great degree of probability that the part which is continuous with the sphenethmoid commissure is the dorsal part of the parieto-

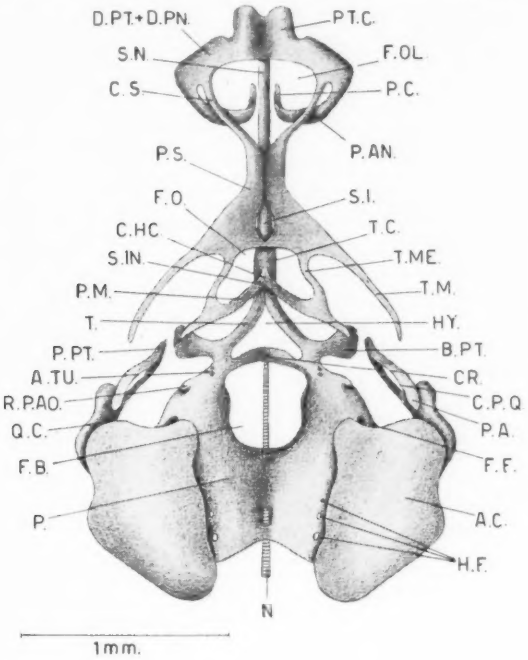

Fig. 1. Graphic reconstruction of the neurocranium of stage I in a dorsal view.

tectal cartilage, and the part which is continuous with the planum antorbitale is the dorsal part of the paranasal cartilage.

Close to the ventral edge of the posterior part of the nasal septum, but quite free from it, the paraseptal cartilage (P.C.) is present. This cartilage has a free anterior end, i.e. it has not yet established connection with the lamina transversalis anterior. The paraseptal cartilage is fused posteriorly with the ventro-medial edge of the planum antorbitale. The sphenethmoid commissure (C.S.) is continuous posteriorly with the anterior edge of the planum supraseptale (P.S.). Thus the two sphenethmoid commissures of the present stage connect the nasal capsule with the planum supraseptale. Similarly in early stages of *Pachydactylus* (Brock, 1932) and *Hemidactylus* (Kamal, in press), the nasal capsule is connected with the planum supraseptale by means of the sphenethmoid commissures. On the other hand, in *Ptyodactylus* (El-Toubi and Kamal) the nasal capsule is never connected with the planum supraseptale.

The nasal septum is continuous posteriorly with the interorbital septum (S.I.). Above the interorbital septum, the planum supraseptale (P.S.) is present. The two halves of the planum have not yet fused together in the middle line, except at their extreme posterior ends. The planum supraseptale is very close to the dorsal edge of the interorbital septum, but the two structures (planum and sep-

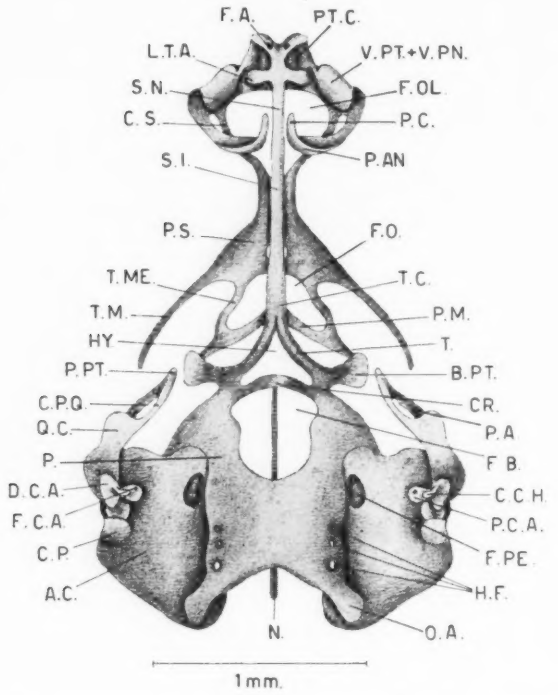

Fig. 2. Graphic reconstruction of the neurocranium of stage I in a ventral view.

tum) are fused together only at their extreme posterior ends. The dorso-lateral edge of the planum supraseptale is continuous posteriorly with the taenia marginalis (T.M.). This taenia extends posteriorly for some distance in a roughly horizontal plane and ends freely, i.e. it is not yet attached with the auditory capsule. Ventro-medial to the taenia marginalis, the planum supraseptale is continuous posteriorly with the taenia medialis (T.ME.). This latter rod extends backwards for some distance and then it is attached with the pila metoptica (P.M.). This pila which is a roughly vertical pillar, extends ventrally and inwards uniting with its fellow of the opposite side forming the subiculum infundibuli (S.IN.) and cartilago hypochiasmatica (C.HC.). This latter cartilage is fused with the dorsal surface of the trabecula communis. Posterior to its attachment with the pila metoptica, the taenia medialis extends postero-dorsally for a very short distance, and ends freely, without being connected to either the taenia marginalis or the parachordal plate. Thus both the pilae accessoria and antotica are absent. However, there is a small process (Figs. 1 & 3, R.P.AO.) attached to the dorso-lateral edge of the anterior part of the parachordal plate, representing the connection between the pila antotica and the plate.

The trabeculae cranii (T.) gradually approach one another forwards till they

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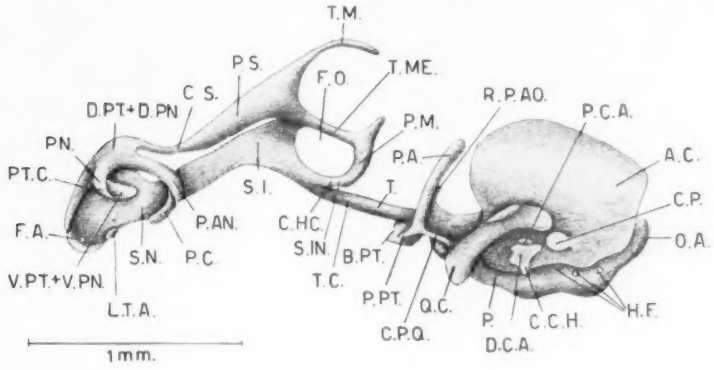

Fig. 3. Graphic reconstruction of the neurocranium of stage I in a lateral view.

come in contact with each other and then fuse together forming the trabecula communis (T.C.). Posteriorly the trabeculae cranii are fused with the anterior edge of the parachordal plate, so that a triangular fenestra hypophyseos (Figs. 1 & 2, HY.) is formed. At the point of fusion between the trabecula cranii and the parachordal plate, a basitrabecular process (B.PT.) is given off on each side. The parachordal plate (P.) is well chondrified. The basicranial fenestra (Figs. 1 & 2, F.B.) is rather large occupying the anterior half of the parachordal plate. It is separated from the hypophysial fenestra by the crista sellaris (CR.). The abducens nerve passes through the abducens tunnel excavated on the dorsal surface of the anterior part of the parachordal plate (Fig. 1, A.TU.). At the extreme hind edge of the parachordal plate, the notochord is completely embedded. The dorsal covering is rather thin and of slight extent, the notochord soon emerges and runs on the dorsal surface of the parachordal plate, with its ventral three quarters embedded in cartilage. The notochord extends anteriorly for some distance in this condition, and then it shifts its position and becomes completely embedded again. Then it traverses the basicranial fenestra, and ends immediately behind the crista sellaris. Thus the notochord does not penetrate the crista sellaris. On the other hand, in stage I of *Hemidactylus* (KAMAL) the notochord penetrates the crista sellaris.

At the present stage of *Tropiocolotes,* three hypoglossal foramina (H.F.) are present on each side of the posterior part of the parachordal plate. This latter plate is continuous postero-laterally with the occipital arch (O.A.) on each side. The arch is quite free from the hind wall of the auditory capsule. Thus the fissura metotica is still open postero-dorsally. At this stage the fissura metotica is a continuous space which has not yet been divided. Also the occipital arch has not yet established connection with the corresponding one of the other side. In other words, the tectum synoticum plus posterius has not yet been formed.

The walls of the anuditory capsules (A.C.) are well chondrified. The foramina

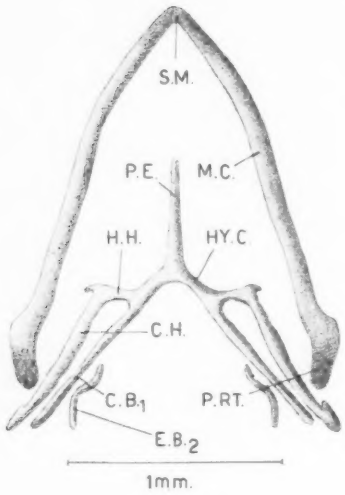

Fig. 4. Graphic reconstruction of the viscerocranium of stage I in a ventral view.

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acustica anterius and posterius are confluent with one another. As the tectum synoticum plus posterius has not yet been formed, the two capsules are still separate from each other. The lateral wall of the auditory capsule bears the crista parotica (C.P.) below and behind the head of the quadrate. The columella auris is formed of a proximal and distal portions which have not yet completely fused with each other. The distal portion is connected with the hyoid arch by means of a thick cartilaginous bar (C.C.H.). The proximal portion is perforated by a minute foramen (Fig. 2, F.C.A.) for the passage of the stapedial artery.

The cartilages of the pterygo-quadrate are continuous with one another. The ventral end of the ascending process (P.A.) is continuous forwards with the posterior pterygoid process (P.PT.) which is very small. The quadrate cartilage is connected with the ascending process by means of a fine cartilaginous bar (C.P.Q.). The basal process (meniscus pterygoideus) and the anterior pterygoid process have not yet chondrified. The two rami of Meckel's cartilage (Fig. 4, M.C.) are attached together anteriorly forming a symphysis meckelii (S.M.). Meckel's cartilage articulates with the quadrate, the processus retroarticularis is present behind the region of articulation. The hyobranchial skeleton assumes its final form. The second branchial arch is represented only by the second epibranchial (E.B.$_2$), i.e. the second ceratobranchial is completely absent.

Stage II [fully formed chondrocranium]

(age: 49 days, total body length: 21.5 mm, Figs. 5—8)

The basicranial fenestra (Figs. 5 & 6, F.B.) has a more or less circular outline. The small process which is observed in the previous stage attached to the dorso-lateral edge of the anterior part of the basal plate completely disappears in the fully formed stage. At the hind end of the basal plate, the notochord is completely embedded. After a short distance, the notochord emerges and runs on the dorsal surface of the parachordal plate with its ventral three quarters embedded in it. The basal plate decreases anteriorly in thickness. Thus the ventral covering decreases gradually until the ventral surface of the notochord is exposed. As a result, in the region of the basal plate which lies just behind the basicranial fenestra, the notochord can be seen in a dorsal and ventral views (Figs. 5 & 6, N.). At the present stage the notochord does not traverse the basicranial fenestra to reach the posterior edge of the crista sellaris, but it ends just anterior to the posterior border of this fenestra. In other words, the anterior part of the notochord has undergone a process of degeneration. While in the previous stage the abducens nerve passes through a tunnel excavated in the basal plate, in the fully formed chondrocranium the tunnel is transformed into a mere groove by the disappearance of its roof. This groove is found on the dorsal surface of the antero-lateral part of the basal plate. On the other hand, in the majority of lizards studied the abducens tunnel persists in the fully formed stage.

The margin of the foramen magnum is pentagonal in shape. In the posterior part of the chondrocranium there is only one tectum, called the tectum synoticum plus posterius (T.S.), the anterior part of which connects the two auditory capsules and its posterior part connects the two occipital arches. The same condition occurs in *Eumeces* (Rice, 1920), *Platydactylus* (Hafferl, 1921), *Calotes* (Ramaswami, 1946), *Mabuya* (Rao and Ramaswami, 1952), *Chalcides* (El-Toubi and Kamal, 1959 a & b), *Ptyodactylus* (El-Toubi and Kamal, in press) and *Hemidactylus* (Kamal, in press). The antero-posterior extension of this tectum is rather small. The processus anterior tecti is completely absent as in other geckos. The number of the hypoglossal foramina (Figs. 6 & 7, H.F.) is three on each side. The occipital condyle possesses two lateral prominences (P.O.CO.) separated from one another by the incisura intercondyloidea (IN.IC.). The notochord is completely embedded in the occipital condyle. The thin cartilaginous layer found above the notochord is continuous with the anterior end of the odontoid process. Thus the connection between the occipital condyle and the odontoid process persists to the fully formed stage. The condyle is formed of the hypocentrum of the proatlas vertebra. The occipitoatlantal (cranio-vertebral) joint is intravertebral and intersegmental.

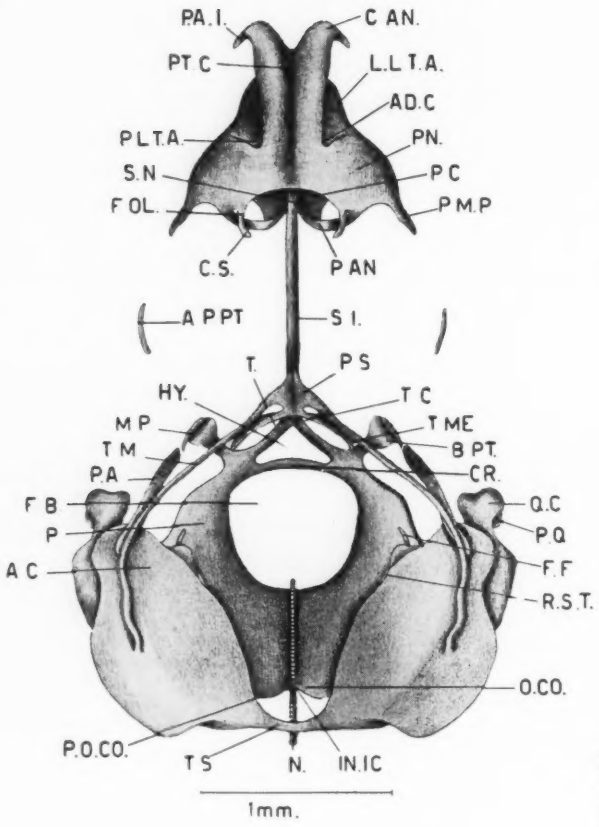

Fig. 5. Graphic reconstruction of the neurocranium of stage II in a dorsal view.

There is only one commissure, known as the anterior basicapsular commissure, between the auditory capsule and the lateral edge of the basal plate. Below the prominentia semicircularis lateralis the crista parotica (Figs. 6 & 7, C.P.) is located. Intercalated between the crista parotica and the head of the quadrate, the processus paroticus (P.P.) is observed. On the medial surface of the auditory capsule, the foramen acusticum anterius, foramen acusticum posterius and foramen endolymphaticum are present. The large foramen perilymphaticum (Fig. 6, F.PE.) is present on the ventral surface of the cavum cochleare. Laterally the fenestra ovalis (Fig. 7, F.OV.), which has an oval outline, is present. The facial foramen (F.F.) is relatively large. The greater part of this foramen is located anterior to the cochlear portion of the auditory capsule. This is probably due to the fact that the forward extension of the cochlear portion is very slight in *Tropiocolotes*. The fissura metotica is completely divided into two parts: an anterior recessus scalae tympani (R.S.T.) for the passage of the glossopharyngeal nerve, and a posterior foramen jugulare (Fig. 6, F.J.) for the passage of the vagus and

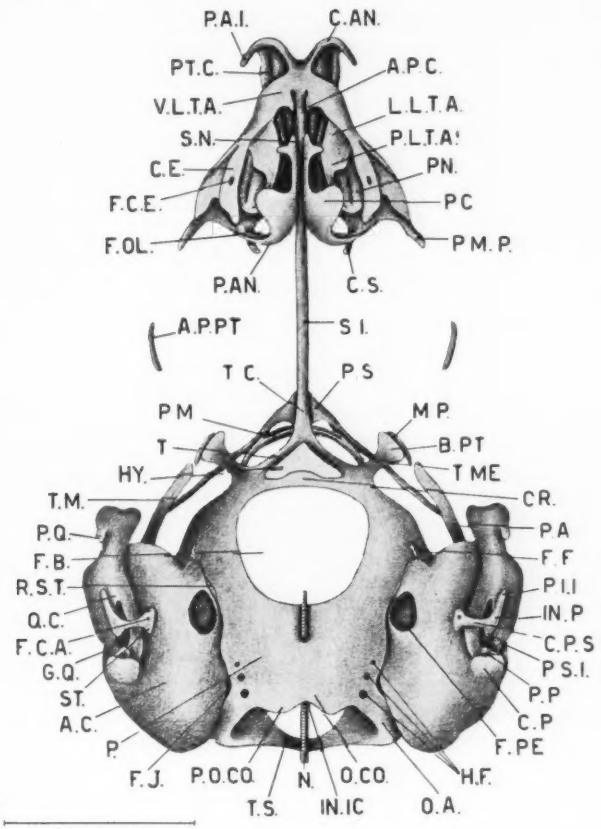

Fig. 6. Graphic reconstruction of the neurocranium of stage II in a ventral view.

spinal accessory nerves. The apertura medialis recessus scalae tympani is not divided into an anterior and posterior portions. On the other hand, in *Ptyodactylus* (El-Toubi and Kamal) and *Hemidactylus* (Kamal) the apertura medialis is divided into an anterior portion and a posterior one by the connection between the medial border of the foramen perilymphaticum and the lateral edge of the parachordal plate.

The columella auris is composed proximally of a stapes or footplate (Figs. 6 & 7, ST.), and distally of an insertion plate (IN.P.). The two parts are attached together by a cartilaginous rod (Fig. 6, C.P.S.). The four processes of the insertion plate are present: the pars inferior (Figs. 6 & 7, P.I.I.) projecting anteriorly, the pars superior (Figs. 6 & 7, P.S.I.) projecting posteriorly, the processus accessorius anterior (Fig. 7, P.AC.A.) which is directed dorsally towards the quadrate without being connected to it, and the processus accessorius posterior or processus interhyalis (Fig. 7, P.AC.P.) which is rudimentary and directed ventrally towards

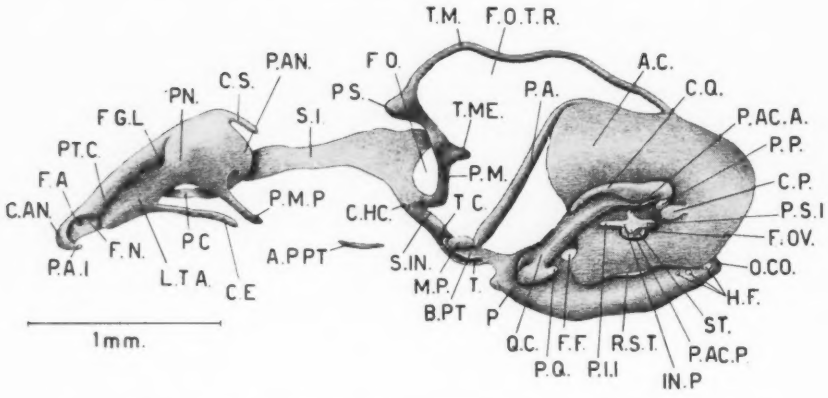

Fig. 7. Graphic reconstruction of the neurocranium of stage II in a lateral view.

the ceratohyal but separate from it. Thus the connection between the columella and the hyoid arch, observed in early stages, is lost in the fully formed chondrocranium. Both the processus internus and processus dorsalis are completely absent. The stapes is perforated by a foramen (Fig. 6, F.C.A.) for the passage of the stapedial artery. This foramen is present in *Lygodactylus* (Brock, 1932) and *Hemidactylus* (Kamal). On the other hand, in *Ptyodactylus* (El-Toubi and Kamal) there is no such foramen.

The trabeculae cranii (T.) which are fused posteriorly with the basal plate, extend anteriorly and medially till they are fused together forming the trabecula communis (T.C.). Each basitrabecular or basipterygoid process (B.PT.) extends off antero-laterally from the point of junction between the posterior end of the trabecula cranii and the basal plate. Between the basitrabecular process and the base of the ascending process, the meniscus pterygoideus (M.P.) is located, representing the basal process of the pterygoquadrate. The trabecula communis is continuous anteriorly with the interorbital septum (S.I.) The planum supraseptale (P.S.) is present above the septum. This latter structure is greatly reduced as compared with that of other lizards. Its two halves are completely fused together in the middle line. The planum supraseptale has a free anterior end. In other words, it is not continuous with the sphenethmoid commissures, and the distance between them is very great. Thus the connection between the nasal capsule and the planum supraseptale, found in the previous stage, is broken in the fully formed chondrocranium. The ventral edge of the planum is free from the dorsal edge of the interorbital septum, except at its extreme posterior end. The taenia marginalis connects the dorso-lateral edge of the planum supraseptale with the roof of the auditory capsule. Ventro-medial to the taenia marginalis, the taenia medialis (T.ME.) connects the posterior edge of the planum supraseptale with the pila metoptica (Figs. 6 & 7, P.M.). This latter rod extends ventrally

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and medially fusing with its fellow of the opposite side forming the subiculum infundibuli (Fig. 7, S.IN.). This latter structure is continued forwards as the cartilago hypochiasmatica (Fig. 7, C.HC.) which fuses with the dorsal edge of the anterior part of the trabecula communis, near the point where the trabecula is continuous with the interorbital septum. It is interesting to notice that the pila metoptica is located exactly below the anterior part of the taenia medialis, and thus it cannot be seen in a dorsal view. Consequently, the fenestra optica cannot be observed in a dorsal or ventral views of the chondrocranium; it is only seen in a lateral view (Fig. 7, F.O.). The taenia medialis is not connected with either the taenia marginalis or the basal plate. Thus the pila accessoria and pila antotica are completely absent. Consequently, one fenestra in the orbitotemporal region is completely delimited; the fenestra optica. The three other fenestrae of this region are completely confluent together.

The nasal septum (S.N.) is unfenestrated, but at the end of its anterior two thirds there is a gap separating the dorsal part of the septum from its ventral part. As the dorsal part terminates posteriorly with a free end, the nasal septum is represented posteriorly by its ventral part. The two cupolae anteriores (C.AN.) form the anteriormost part of the nasal capsule. Each cupola is perforated medially by the foramen apicale for the passage of the ramus medialis of the ethmoid nerve. Thus this foramen can be seen in a lateral view of the chondrocranium, through the fenestra narina (Fig. 7, F.A.). The parietotectal cartilages (PT.C.) form the roof as well as the anterior part of the side wall of the nasal capsule. As the fenestra superior is completely absent, the roof of the capsule is complete. At the anterior border of the fenestra narina (Fig. 7, F.N.) there is the conspicuous processus alaris inferior (P.A.I.). The processus alaris superior is completely absent. As the parietotectal cartilage comes into contact with the lamina transversalis anterior, the fenestra narina is completely closed posteriorly. Thus the fissura lateralis nasi is completely absent. As a result, a complete zona annularis is present. On the other hand, in the fully formed chondrocranium of *Platydactylus* (Hafferl, 1921), *Ptyodactylus* (El-Toubi and Kamal) and *Hemidactylus* (Kamal), the parietotectal cartilage is separated from the lamina transversalis anterior by the fissura lateralis nasi.

Posteriorly, the parietotectal cartilage is separated from the lamina transversalis anterior by a foramen (Fig. 7, F.G.L.) through which the duct of the lateral nasal gland passes to open into the nasal sac. While the anterior part of this foramen lies in front of the concha nasalis, its posterior part is located at the same level of the anterior end of the concha. In *Ptyodactylus* (El-Toubi and Kamal) this foramen is present on the medial wall of the concha nasalis. In *Hemidactylus* (Kamal) it is located anterior to the concha. Thus it can be stated that in *Tropiocolotes,* the foramen for the duct of the lateral nasal gland occupies an intermediate position between the foramen of *Ptyodactylus* and that of *Hemidactylus.* The lamina transversalis anterior is composed of two lamellae which are con-

tinuous with one another, a ventral lamella and a lateral one. The lateral lamella is continuous posteriorly with a process which forms a part of the wall of the concha nasalis. The posterior edge of the ventral lamella of the lamina transversalis anterior is fused medially with the paraseptal cartilage and laterally with the ectochoanal cartilage. The paraseptal cartilage (P.C.) runs very close to, but free from, the ventral edge of the nasal septum, and is fused posteriorly with the ventro-medial edge of the planum antorbitale. In *Tropiocolotes,* the paraseptal cartilage is not in the form of a continuous plate. This is due to the fact that the anterior end of the paraseptal cartilage (Fig. 6, A.P.C.), which is connected with the lamina transversalis anterior, is separated from its main part. The ectochoanal cartilage (C.E.) is perforated nearly at its middle length by a small foramen (Fig. 6, F.C.E.) through which a small nerve passes. This nerve is a branch of the maxillary nerve.

The side wall of the posterior part of the nasal capsule, which is located more laterally than the side wall of the anterior part of the capsule, is represented by the paranasal cartilage (PN.). Because of the absence of the fenestra lateralis, this cartilage is complete. In *Eumeces* (Rice, 1920), *Lygosoma* (Pearson, 1921), *Calotes* (Ramaswami, 1946), *Chalcides* (El-Toubi and Kamal, 1959 b) and *Hemidactylus* (Kamal), the fenestra lateralis is also absent. On the other hand, in *Lacerta* (Gaupp, 1900 and de Beer, 1930) the fenestra lateralis is present. The concha nasalis is formed medial to the recessus extraconchalis. The posterior process of the lamina transversalis anterior forms the ventral wall of the concha. Its dorso-lateral wall is formed of the inner wall of the paranasal cartilage. Its dorso-medial wall is formed of the parietotectal cartilage. Just posterior to the aditus conchae, the ventral wall of the concha is connected with its lateral wall. Thus it can be stated that the cavity of the anterior part of the concha nasalis is completely encircled on all sides by cartilage. The extension of this complete enclosure is rather slight, and soon a crevice is found at the ventro-lateral corner of the concha. This crevice increases in size in the posterior direction till it occupies the greater part of the ventral surface of the concha. In the case of *Ptyodactylus* (El-Toubi and Kamal, in press) and *Hemidactylus* (Kamal), the concha nasalis is open ventro-laterally throughout its entire length.

The paranasal cartilage passes posteriorly into the lamina orbitonasalis or planum antorbitale (P.AN.) which forms the hind wall of the nasal capsule. There is no contact between the planum antorbitale and the dorsal edge of the nasal septum, although they are very close to each other. The ventro-lateral edge of the planum antorbitale is continued backwards into the processus maxillaris posterior (P.M.P.). There is no palatine projection of the processus maxillaris posterior. Similar to other geckos, the processus maxillaris anterior is completely lacking.

In the fully formed chondrocranium of *Tropiocolotes,* the sphenethmoid commissures are rather short. They are quite separate from the anterior end of the

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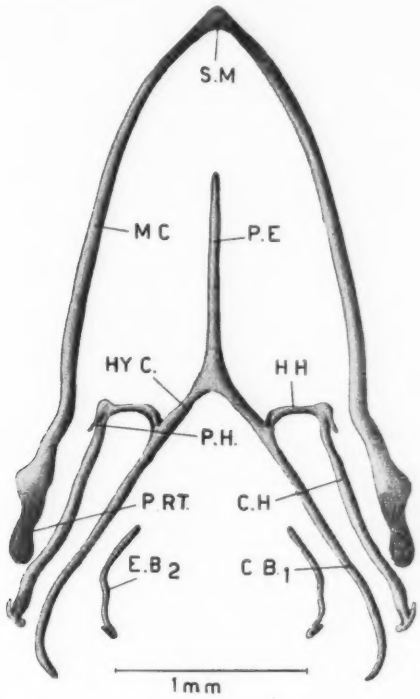

Fig. 8. Graphic reconstruction of the viscerocranium of stage II in a ventral view.

planum supraseptale and the distance between them is remarkable. Thus the connection between the nasal capsule and the planum supraseptale, present in the previous stage, is lost in the fully formed chondrocranium. As a result of the reduction of the sphenethmoid commissure, the fenestra olfactoria evehens has no posterior boundary. Consequently, the olfactory nerve and the profundus branch of the trigeminal nerve pass in conjunction at the dorsal surface of the planum antorbitale.

The four processes of the pterygoquadrate (otic, ascending, pterygoid and basal) are present. The quadrate (Q.C.) possesses a conspicuous projection (P.Q.), posterior to its articulation with Meckel's cartilage, which is directed backwards and ends freely. The postero-dorsal end of the processus ascendens (P.A.) is completely fused with the anterior wall of the auditory capsule. In the case of *Ptyodactylus* (El-Toubi and Kamal) and *Hemidactylus* (Kamal) there is contact between the processus ascendens and the auditory capsule, but there is no fusion. The posterior pterygoid process, present in the previous stage, disappears completely in the fully formed chondrocranium. The anterior pterygoid process (A.P.PT.) is in the form of a slender separate cartilaginous rod. The meniscus pterygoideus (M.P.) represents the detached basal process.

In the hyobranchial skeleton (Fig. 8), three visceral arches are present: the

hyoid, first and second branchial arches. The hind end of the ceratohyal is not fused with the crista parotica of the auditory capsule. Thus it can be stated that there is no cartilaginous connection between the hyoid arch and the auditory capsule. The same condition occurs in *Hemidactylus* (KAMAL). On the other hand, in the case of *Lygodactylus,* (BROCK, 1932) and *Ptyodactylus* (EL-TOUBI and KAMAL), the posterior end of the ceratohyal is fused with the auditory capsule. The second branchial arch is represented only by the second epibranchial ($E.B._2$), i.e. the second ceratobranchial is completely lacking.

Summary

1—The chondrocranium of the gecko, *Tropiocolotes tripolitanus* has been studied in two developmental stages; stage I which is a moderate stage and stage II which possesses the fully formed chondrocranium.

2—In stage I, the sphenethmoid commissures are continuous posteriorly with the planum supraseptale. The parietotectal and paranasal cartilages are fused together. The pila accessoria is completely absent and the pila antotica is represented by a rudimentary process attached to the parachordal plate.

3—In the fully formed chondrocranium, the abducens tunnel is transformed into a groove. The apertura medialis of the recessus scalae tympani is not divided into an anterior and posterior portions. The stapes of the columella is perforated by a foramen for the passage of the stapedial artery. The planum supraseptale is greatly reduced. Due to the fact that the pila metoptica is located exactly below the anterior part of the taenia medialis, the fenestra optica cannot be seen in a dorsal or ventral views. The pilae accessoria and antotica are completely absent.

4—In the nasal capsule, the fenestrae superior and lateralis are completely absent. No fissura lateralis nasi can be observed between the parietotectal cartilage and lamina transversalis anterior. The anterior end of the paraseptal cartilage is separated from its main part. The sphenethmoid commissures are short. The olfactory nerve and the profundus branch of the trigeminal nerve pass in conjunction at the dorsal surface of the planum antorbitale.

5—The postero-dorsal end of the processus ascendens is completely fused with the anterior wall of the auditory capsule. The hind end of the hyoid arch does not fuse with the auditory capsule. The second ceratobranchial is absent.

Abbreviations to figures

A.C., auditory capsule
AD.C., aditus conchae
A.P.C., anterior end of paraseptal cartilage
A.P.PT., anterior pterygoid process
A.TU., abducens tunnel
B.PT., basitrabecular (basipterygoid) process
C.AN., cupola anterior

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C.B.1 first ceratobranchial
C.C.H., cartilaginous bar connecting distal portion of columella auris with hyoid arch
C.E., ectochoanal cartilage
C.H., ceratohyal
C.HC., cartilago hypochiasmatica
C.P., crista parotica
C.P.Q., cartilaginous bar connecting processus ascendens with quadrate
C.P.S., cartilaginous rod connecting insertion plate with stapes
C.Q., crest of quadrate
CR., crista sellaris
C.S., sphenethmoid commissure
D.C.A., distal portion of columella auris
D.PT.+D.PN., dorsal part of parietotectal cartilage plus outer dorsal part of paranasal cartilage
E.B.2, second epibranchial
F.A., foramen apicale
F.B., fenestra basicranialis
F.C.A., foramen in columella auris
F.C.E., foramen in ectochoanal cartilage
F.F., facial foramen
F.G.L., foramen for duct of lateral nasal gland
F.J., foramen jugulare
F.N., fenestra narina
F.O., fenestra optica
F.OL., fenestra olfactoria
F.O.T.R., fenestra of orbitotemporal region representing the confluent fenestrae epioptica, metoptica and prootica
F.OV., fenestra ovalis
F.PE., foramen perilymphaticum
G.Q., gap in quadrate
H.F., hypoglossal foramina
H.H., hypohyal
HY., fenestra hypophyseos
HY.C., hyoid corpus
IN.IC., incisura intercondyloidea
IN.P., insertion plate of columella
L.L.T.A., lateral lamella of lamina transversalis anterior
L.T.A., lamina transversalis anterior
M.C., Meckel's cartilage
M.P., meniscus pterygoideus
N., notochord
O.A., occipital arch
O.CO., occipital condyle
P., parachordal plate
P.A., processus ascendens
P.AC.A., processus accessorius anterior of columella auris
P.AC.P., processus accessorius posterior of columella auris
P.A.I., processus alaris inferior
P.AN., planum antorbitale
P.C., paraseptal cartilage
P.C.A., proximal portion of columella auris
P.E., processus entoglossus
P.H., process present at the point of fusion between ceratohyal and hypohyal
P.I.I., pars inferior of insertion plate
P.L.T.A., posterior process of lamina transversalis anterior
P.M., pila metoptica
P.M.P., processus maxillaris posterior
PN., paranasal cartilage
P.O.CO., lateral prominence of occipital condyle
P.P., processus paroticus
P.PT., posterior pterygoid process
P.Q., projection of quadrate
P.RT., processus retroarticularis
P.S., planum supraseptale
P.S.I., pars superior of insertion plate of columella auris
PT.C., parietotectal cartilage
Q.C., quadrate cartilage
R.P.AO., process representing the connection between pila antotica and basal plate
R.S.T., recessus scalae tympani
S.I., interorbital septum
S.IN., subiculum infundibuli
S.M., symphysis meckelii
S.N., nasal septum
ST., stapes of columella auris
T., trabecula cranii
T.C., trabecula communis
T.M., taenia marginalis
T.ME., taenia medialis
T.S., tectum synoticum plus posterius
V.L.T.A., ventral lamella of lamina transversalis anterior
V.PT.+V.PN., ventral part of parietotectal cartilage plus inner ventral part of paranasal cartilage

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"Chromaffin" cells in the heart of *Myxine glutinosa*

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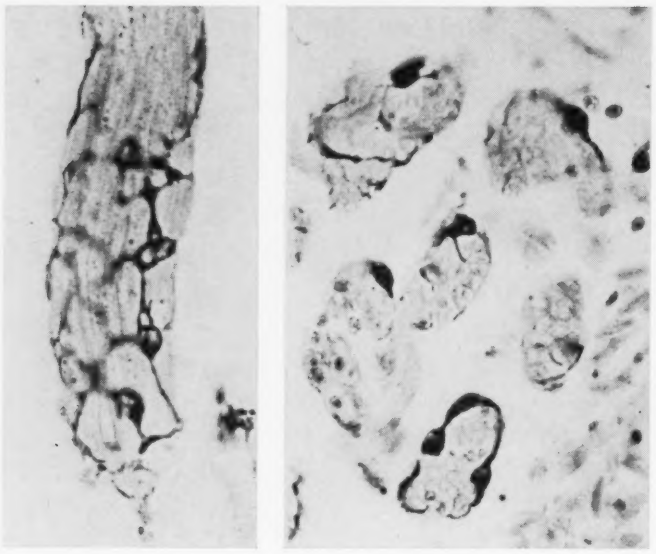
GASKELL (1912) observed chromaffin cells in the sinus venosus of the lamprey heart. Cells of the same type are also present in the auricle and the ventricle of *Lampetra* (AUGUSTINSSON, FÄNGE, JOHNELS & ÖSTLUND 1956). These observations were made on preparations stained with silver according to the Bodian protargol method (BODIAN 1936, 1937, JOHNELS 1955). With this method no cells of a similar type could be detected in the heart of *Myxine*.

Preparations made according to a new silver staining method (PALMGREN 1960, formula "I_1 24 hours D_1 rep.") have proved that cells of a type similar to the chromaffin cells of *Lampetra* also exist in the heart of *Myxine*.

As in the *Lampetra* heart the cells are distributed over the interior surface of the muscular bundles of the heart of *Myxine*. In the protargol preparations of *Lampetra* (se AUGUSTINSSON, FÄNGE, JOHNELS & ÖSTLUND 1956) the cell processes are indistincly stained and in the periphery they are usually not stained at all. In the present preparations of the heart of *Myxine* not only the cell bodies but also the cell processes are clearly seen (Fig. 1). As in *Lampetra* they connect with each other in such a way that a continuous system of these cells is formed throughout the heart. The cell bodies are mostly located at some distance from each other but they may also form small groups in some places. The cells seem to be as frequent in the ventricle as in the auricle. They are smaller than the chromaffin cells of a heart of similar size of *Lampetra fluviatilis*, the cells of which are larger than those of *L. planeri*. The nuclei are slightly ovoid in shape; they are distinct although weakly stained in the preparations. The plasm of the chromaffin cells of the lamprey heart is usually granular in the protargol preparations. The same is true of the present preparations of the heart of *Myxine*. The plasm of the cell processes is also granular in *Myxine*.

The heart of *Lampetra* contains 4—5 times more catechol amines per gram than the *Myxine* heart. However the hearts of both contain strikingly large quantities of catechol amines in comparison with the hearts of other vertebrates (AUGUSTINSSON, FÄNGE, JOHNELS & ÖSTLUND 1956). Although it is possible that the heart muscles of the cyclostomes may contain these substances (AUGUSTINSSON, FÄNGE, JOHNELS & ÖSTLUND 1956), it seems natural to ascribe such a content to cellular elements of a special nature. The similarities between the particular cells



A B

Fig. 1. "Chromaffin cells in the heart of *Myxine glutinosa*. A, longitudinal section, B, transverse section of bundle of heart muscles. 425 ×.

of the heart of lampreys and of the heart of hagfishes indicate that the cells are of the same nature and, thus, that the cells of *Myxine* may be "chromaffin" cells.

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